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THE PASSAGE OF NEUTRALIZING SUBSTANCES FROM THE BLOOD INTO THE CEREBROSPINAL FLUID IN POLIOMYELITIS.

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Recovery in poliomyelitis as in other infectious diseases is accomplished through a process of active immunization. The immunity substances on which recovery depends have been detected by neutralization[1] and protection[2] tests, but not by other biological methods. Similarly, monkeys in which experimental poliomyelitis is induced by inoculation yield corresponding immunity bodies. Evidence exists to show that the neutralizing principles arise irrespective of the intensity of the clinical symptoms,[3] and apparently as early as the 6th day of the disease (page 501). Similar conditions exist in respect to the experimental disease in monkeys. Both protective and neutralizing principles have been discovered in animals which have passed through an attack induced by inoculation of filterable virus from human or from monkey sources.

The manner in which the immunity principles act in arresting the pathologic processes has become of especial interest on account of the treatment of cases of epidemic poliomyelitis by intraspinal injections of immune human and other sera. This method is based on the therapeutic experiments of Flexner and Lewis[4] with monkeys, the results of which were later extended to human beings by Netter and his coworkers[5] and since by many others.[6,7,8]

[1] Levaditi and Landsteiner, *Compt. rend. Soc. biol.*, 1910, lxviii, 311.

[2] Flexner, S., and Lewis, P. A., *J. Am. Med. Assn.*, 1910, liv, 45.

[3] Netter, A., and Levaditi, C., *Compt. rend. Soc. biol.*, 1910, lxviii, 855.

[4] Flexner and Lewis, *J. Am. Med. Assn.*, 1910, liv, 1780; lv, 662.

[5] Netter, A., Gendron, A., and Touraine, *Compt. rend. Soc. biol.*, 1911, lxx, 625. Netter, A., *Bull. Acad. mêd.*, 1915, lxxiv, series 3, 403. Netter, A., and Salanier, M., *Bull. et mêm. Soc. mêd. hôp. Paris*, 1916, xl, series 3, 299.

[6] *Weekly Bull. Dept. Health, City of New York*, 1916, N. S. v, 345.

[7] Fischer, L., *Med. Rec.*, 1917, xci, 52.

[8] Amoss, H. L., and Chesney, A. M., *J. Exp. Med.*, 1917, xxv, 581.

Recently, however, both normal human serum[6] and normal horse serum[9] have been employed for intraspinal injection. It is still too early to state whether these latter sera exerted any definite influence on the pathologic processes.

All sera introduced into the subarachnoid spaces act as foreign bodies, and if sterile give rise to aseptic inflammation. The severity of the inflammation is less with homologous, than with heterologous serum. But experiments have shown that in monkeys very slight changes in the permeability of the meninges allow infection to take place after intravenous injection of the virus which otherwise is incapable of causing infection.[10]

Hence we have attempted to detect in the cerebrospinal fluid immunity principles such as exist in the blood. In the course of other experiments carried out by us[11] at a much earlier period, neutralization tests were made with the cerebrospinal fluid. Seven fluids were tested, of which only one proved neutralizing. This specimen came from a convalescent child paralyzed 6 weeks earlier. No examination was made at the time to discover whether the fluid indicated persistence of the inflammatory processes in the meninges. The conclusion reached was to the effect that, while possible, it was unusual for neutralizing principles to be contained in the cerebrospinal fluid during convalescence from epidemic poliomyelitis. Incidentally, it was determined that neutralizing bodies were not produced locally.

The present inquiry has arisen from the idea that under certain circumstances immunity substances enter the cerebrospinal fluid from the blood and assist materially in the healing process. If this supposition is founded on fact, we might view the inflammatory conditions occurring in the meninges, which increase their permeability to circulating proteins otherwise excluded,[12] as beneficial; and from this it may follow that any advantage actually shown to be derived from the intraspinal administration of normal human or horse serum

[9] Sophian, A., *J. Am. Med. Assn.*, 1916, lxvii, 426.

[10] Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1917, xxv, 525.

[11] Flexner, S., Clark, P. F., and Amoss, H. L., *J. Exp. Med.*, 1914, xix, 205.

[12] Mott, F. W., *Lancet*, 1910, ii, 79. Flexner, S., *J. Am. Med. Assn.*, 1913, lxi, 447, 1872.

may be the result not of the effects of the serum as such, but of a further increase in this permeability.

Neutralization Tests with Cerebrospinal Fluid.

Three preliminary tests were made with human cerebrospinal fluid and one sample of human blood. Two of the former were drawn on the 2nd and 18th days, respectively, of the disease. One of them contained 920 cells per c. mm., and gave a positive test for globulin; the other contained 215 cells, and also gave a positive test for globulin. 2 cc. of each fluid were added to 0.1 cc. of filtrate of active monkey spinal cord, the mixture was incubated for 2 hours at 37°C., kept at 4°C. for 16 hours, and then inoculated intracerebrally, under ether anesthesia, into two *Macacus rhesus* monkeys. One monkey became paralyzed on the 6th, and the other on the 10th day. Both died. The third test with cerebrospinal fluid was made with an equal mixture of two specimens, one taken on the 6th, and the other on the 8th day of illness. Both contained an excess of cells and globulin. The inoculated monkey became paralyzed on the 7th day and afterwards developed contractures. It was etherized for histological study 6 weeks after inoculation. Hence none of the specimens tested contained demonstrable neutralizing substances.

The blood test was made with human serum obtained on the 6th day of illness. 1 cc. was mixed with 0.1 cc. of filtrate of active monkey virus, incubated, and kept as described above. The mixture was injected intracerebrally[13] into a *Macacus rhesus*. It remained well. Hence it appears that adequate neutralizing substances were present in the specimen.

The next experiment was made in order to determine whether the neutralizing substances may be made to pass from the blood into the cerebrospinal fluid. For this purpose normal monkeys were chosen. Each animal was given an intraspinal injection of normal horse serum and 16 hours later an intravenous injection of immune monkey serum from recovered poliomyelitic monkeys. The cerebrospinal fluid was withdrawn at different periods and tested for neutralizing properties. Every care was exercised to avoid admixture with blood. When

[13] Ether anesthesia was employed for the intracerebral inoculations.

blood appeared in the puncture needle, the fluid was discarded and the monkey yielding it was not used again for that experiment.

Experiment 1.—Dec. 5, 1916. 5 p.m. 2 cc. of normal horse serum were injected intraspinally into a *Macacus rhesus*. Dec. 6, 9.10 a.m. Injected 10 cc. of immune monkey serum intravenously. The cerebrospinal fluid was withdrawn 6, 9, and 24 hours later. The 6 and 9 hour specimens were combined in order to produce a total of 1 cc. Two neutralization tests were performed.

Monkey A.—Dec. 8. A *Macacus rhesus* received intracerebrally a mixture consisting of 0.1 cc. of active filtrate of poliomyelitic virus and 1 cc. of the combined 6 and 9 hour cerebrospinal fluids which had been incubated for 10 hours at 37°C. and kept for 3 hours at 4°C. No symptoms developed.

Monkey B.—Dec. 8. This test was repeated with the 24 hour specimen of cerebrospinal fluid. Dec. 18. Animal excitable and slow; protects right arm; ataxic. Dec. 19. Arms paralyzed; legs and back weak. Dec. 20. Prostrate. Dec. 21. Etherized.

Autopsy showed marked lesions of poliomyelitis in the spinal cord.

In these experiments the fluids withdrawn at the 6th and the 9th hours exerted a neutralizing effect, while the 24 hour specimen did not. The test was repeated as follows:

Experiment 2.—Jan. 23, 1917. 4 p.m. 2 cc. of normal horse serum were ininjected intraspinally into a *Macacus rhesus*. Jan. 24, 8.45 a.m. Injected intravenously 10 cc. of immune monkey serum. The cerebrospinal fluid was withdrawn 6 and 9 hours later and combined. One neutralization test was made with active filtered virus.

Monkey C.—Jan. 26. A *Macacus rhesus* received intracerebrally a mixture consisting of 0.1 cc. of filtrate virus and 1 cc. of combined spinal fluid incubated at 37°C. for 2 hours and kept at 4°C. for 15 hours. No symptoms developed.

This experiment confirms the previous one. On the other hand, in one test with fluid withdrawn at the 6th hour, neutralization was not effected. The protocol follows.

Experiment 3.—The procedure was identical with the two preceding experiments, except that the puncture was made at the end of 6 hours only. The cerebrospinal fluid was incubated with 0.1 cc. of filtrate virus and injected intracerebrally into a *Macacus rhesus* on Jan. 4, 1917. Jan. 13. Ataxia; widespread muscular weakness. Jan. 14. Prostrate; etherized.

At autopsy typical lesions of poliomyelitis were present.

This series of experiments was controlled with the cerebrospinal fluid withdrawn from normal monkeys which had received an intraspinal injection of horse serum 18 hours before, but which had not received an intravenous injection of immune serum. An illustrative protocol follows.

Experiment 4.—Dec. 23, 1916. A *Macacus rhesus* was injected intracerebrally with an incubated mixture of 0.1 cc. of filtrate virus and 2 cc. of cerebrospinal fluid obtained from two monkeys which 18 hours before had received intraspinal injections of normal horse serum. Dec. 29. Tremor of head; ataxia; facial asymmetry; widespread muscular weakness. Dec. 30. Prostrate. Jan. 5, 1917. Etherized.

At autopsy typical lesions of poliomyelitis were present.

DISCUSSION.

The detection of the neutralizing immunity substances in the cerebrospinal fluid of human beings, at relatively early and later periods in the course of poliomyelitis, has been accomplished so rarely as to constitute a marked exception At first sight, this observation would seem to indicate that the neutralizing principles do not pass into the fluid.

On the other hand, the experiments with passively immunized monkeys show unmistakably that the neutralizing substances are capable of passing into the cerebrospinal fluid under conditions in which the meninges have been inflamed. Later, however, when the inflammation is subsiding, the neutralizing substances either do not pass into the cerebrospinal fluid in detectable amounts or are fixed by the nervous tissues. However, cognizance must also be taken of the possibility in lightly passively immunized animals that the falling concentration of the immune bodies in the blood due to elimination may explain their failure to pass into the cerebrospinal fluid after the 9th hour.

While this point has not been settled, it is probable that in actively immune animals the passage of the neutralizing substances from the blood into the cerebrospinal fluid would continue as long as the inflammation present in the meninges rendered the structures easily permeable to the protein constituents of the blood. Probably the human fluids investigated by us were taken either at too early a period (2nd

day of the disease) or at too late a period (after the meningeal inflammation had subsided) to yield neutralizing effects. Until sufficient time has elapsed to permit the active immunization of the body to occur, no neutralizing substances are, of course, available. We have recorded an instance in which on the 6th day of illness the blood contained these substances in detectable amounts. Moreover, once the meningeal lesions are healed, these substances would no longer pass through. If, therefore, the healing process is inaugurated or facilitated by the passage of immunity substances from the blood into the cerebrospinal fluid, the height of that process would probably come in the first week of the illness.

What the experiments show conclusively is that once the neutralizing substances are contained within the blood, they can be made to pass into the cerebrospinal fluid by the production of an aseptic meningitis. We know that the injection of immune serum intraspinally tends to arrest the pathologic poliomyelitic process in monkeys and apparently are learning that it performs this purpose also in man. It is desirable, therefore, to consider what reason may exist for the therapeutic employment of non-immune sera, such as normal human and normal horse serum, in the treatment of cases of human poliomyelitis.

As far as present results can be interpreted, the therapeutic value of immune serum is largely confined to the early period of the disease corresponding with its onset.[8] The value is much less definitely shown, although not excluded, for the later stages when paralysis has already appeared and is extending. It is during the early period that the immunity processes are still in abeyance and that the blood is probably devoid of neutralizing substances. Hence no diversion into the cerebrospinal fluid could be accomplished at that time by increasing the permeability of the meninges through injections of normal serum. It is not easy to see what benefit may be expected at this most favorable time by the injection of normal serum, while it is obviously rational to employ an immune serum. Whether at later periods, when the immunity response has already taken place, the normal serum may hold out more promise cannot be stated; but as even immune serum is far less effective then, the promise seems to be small.

CONCLUSIONS.

The cerebrospinal fluid taken very early and quite late in the course of acute poliomyelitis exhibits no neutralizing action on filtered poliomyelitic virus.

The blood serum on the 6th day of the disease already contains the neutralizing principles.

The injection of sterile horse serum into the cerebrospinal meninges in monkeys increases their permeability, so that they permit the immunity neutralizing principles passively injected into the blood to pass into the cerebrospinal fluid.

The passage in passively immunized monkeys takes place during a relatively brief space of time and apparently only while the inflammatory reaction produced by the horse serum is at its height.

It is established for monkeys and rendered probable for man that the intraspinal injection of immune serum in poliomyelitis is curative. In monkeys normal serum exerts no such action, and at present nothing can be stated definitely regarding the therapeutic effect of normal serum in man except that probably any benefits which may arise from its employment would be attributable not to the action of the serum as such, but to the escape of circulating immunity principles in the blood made possible by the aseptic inflammation set up by it in the meninges.

As the immunity principles appear in the blood only after several days, and the reported favorable effects of the immune serum treatment relate to the first days of illness, the employment of normal serum is thus not indicated, while that of an immune serum is.

NEUTRALIZATION OF THE VIRUS OF POLIOMYELITIS BY NASAL WASHINGS.

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(Received for publication, January 25, 1917.)

The occurrence of the infectious microorganism of poliomyelitis—the virus, so called—in the mucous membranes of the nasopharynx and in their secretions is now firmly established.[1,2] Not only may the virus be demonstrated by inoculation tests during the acute period[3] of the disease, but it is known to persist there in some cases for many months after convalescence[4,5] and, conversely, it has been detected in certain instances in the washings from the nasopharynx of healthy persons who have been in intimate contact with the acutely ill.[6] Finally, the fact has been determined by experiment that when the virus is introduced directly into the central nervous tissues wherein it multiplies, it also appears in the mucous membranes of the nose and throat. These facts indicate that the nasopharyngeal mucous membranes play an important part in the pathology of epidemic poliomyelitis; and the weight of opinion today is to the effect that the ingress and egress of the virus take place by way of these structures.

One of the most important questions arising out of the data presented above is that relating to the so called healthy carriers of the

* Maintained by a special fund privately donated.

[1] Flexner, S., and Lewis, P. A., *J. Am. Med. Assn.*, 1910, liv, 535.

[2] Landsteiner, K., Levaditi, C., and Pastia, C., *Semaine méd.*, 1911, xxxi, 296.

[3] Flexner, S., and Clark, P. F., *J. Am. Med. Assn.*, 1911, lvii, 1685. Landsteiner, Levaditi, and Danulesco, *Compt. rend. Soc. biol.*, 1911, lxxi, 558.

[4] Lucas, W. P., and Osgood, R. B., *J. Am. Med. Assn.*, 1913, lx, 1611.

[5] Kling, C., Pettersson, A., Wernstedt, W., and Josefson, A., *Communications Inst. méd. État à Stockholm*, 1912, iii.

[6] Flexner, S., Clark, P. F., and Fraser, F. R., *J. Am. Med. Assn.*, 1913, lx, 201. Kling, C., and Pettersson, A., *Deutsch. med. Woch.*, 1914, xl, 320.

virus, and for two main reasons. The healthy carriers may be the means of transporting the virus to other persons less resistant who may develop poliomyelitis; or the carrier, healthy when first contaminated, may subsequently develop the infection. At present the means at our disposal for studying the subject of virus carriers are so imperfect that no adequate notion of their number and distribution can be obtained. As long as the inoculation of monkeys with the washings from the nasopharynx must be relied upon to furnish this information, complete knowledge cannot be acquired.

There is, however, another fact which may prove to be significant. Assuming that during the prevalence of epidemics, many persons become contaminated with the virus, the question arises whether this condition need necessarily be either a menace to the contaminated person himself or to others. The answer to this question may lie in the reaction of the secretions of the nasopharyngeal mucous membranes to the virus present upon them. It is possible that in one person the secretions do not exercise a harmful action on the virus, while in another they do. This injurious action upon the virus may be of the nature of a protection to the individual contaminated as well as to the public in general.

It has often been observed that washings made from the nasopharynx may be ineffective when introduced into monkeys, and the lack of power to cause infection has been attributed to insufficient quantity or low infective power of the virus believed to be contained in the secretions removed. No note has been taken of the possibility that the washings are ineffective because the secretions of the mucous membrane are destructive or neutralizing to the virus of poliomyelitis. While this possible action may affect the inoculation tests in cases of acute poliomyelitis, it would be far more likely to be operative in the supposed carrier because of the small amount of virus and the probability of diminished virulence in the latter. Because of these considerations, a series of experiments was carried out to determine (1) the smallest quantity of a standard virus which can be detected in washings, and (2) the action of the washings of different persons upon the virus itself.

Reference has already been made to the fact that nasal washings, of contacts especially, have in a few instances produced polio-

myelitis when injected into monkeys. As the virus obtained directly from human beings possesses low virulence for monkeys, and is injected greatly diluted, the small number of successful inoculations is significant. Tests were made to determine the effect of concentration of washings on the activity of the virus. Amounts of virus which would certainly produce the infection if injected directly were added to a filtered washing fluid obtained from persons not having been exposed to the infection. The mixtures were separately reduced to small volume *in vacuo* at low temperatures and injected into monkeys. The results obtained were variable, for reasons which at first were not obvious, but the tests nevertheless showed that the filtered virus in certain amounts may withstand concentration in washing fluids without losing entirely its infective power.

EXPERIMENTAL.

Experiment 1.—The nasal cavities of a normal adult, H. L., were rinsed thoroughly with 50 cc. of distilled water. The collected fluids were passed through a Berkefeld filter and 0.2 cc. of a Berkefeld filtrate of a 5 per cent suspension of poliomyelitic brain was added. The mixture was reduced *in vacuo* at 37°C. to 4 cc. and injected intracranially, under ether anesthesia, into a *Macacus rhesus*. The monkey became partially paralyzed on the 10th day, completely prostrated on the 12th day, and died on the 14th day. Typical lesions of poliomyelitis were present.

Experiment 2.—The washings of a normal adult, W. T., were obtained in the manner described above. To the filtered fluid was added 0.5 cc. of a Berkefeld filtrate of active virus. The mixture was concentrated at 37°C. *in vacuo* and injected intracranially, under ether anesthesia, into a *Macacus rhesus*. The monkey remained well. As the result of a later protection test the monkey died of poliomyelitis after an appropriate injection of potent virus.

Filtration through Berkefeld or other porcelain filters is undertaken to remove the bacteria always present in the nasal and buccal secretions. But the bacteria can be either killed or their multiplication inhibited by certain antiseptic chemicals which affect to a less extent the virus of poliomyelitis. Thus 0.5 per cent carbolic acid destroys pyogenic bacteria in tissues and leaves the virus intact. Experiments also showed that ether acted more severely on the ordinary bacteria than on the virus. In order, therefore, to obviate any loss of virus which might result from its retention by the

filters, ether was employed to sterilize the washings. Preliminary tests showed that contact of ether for 20 hours with the virus contained in an emulsion of the spinal cord does not destroy it. The test made with washings indicates that while a shorter exposure may not kill all the bacteria, yet they are so greatly diminished that no ordinary infection is produced on inoculation into monkeys. And yet, as the experiments which follow show, while the filtrate contained in 0.8 per cent salt solution is active after the ether treatment, that mixed with the nasal washings is ineffective. The ineffectiveness at first believed to have been due to injury of the virus by the ether or too great dilution of the fluid inoculated, is now probably explicable in other ways.

Experiment 3.—1.8 cc. of a Berkefeld filtrate of active virus were added to 4.2 cc. of isotonic sodium chloride solution and 1 cc. of chemically pure ether. The mixture was shaken for 20 hours at room temperature. The ether was allowed to evaporate and 1 cc. of the remaining mixture, representing 0.3 cc. of virus filtrate, was injected intracranially, under ether anesthesia, into a *Macacus rhesus*. The monkey was almost prostrate on the 7th day, completely prostrate on the 8th, and etherized when moribund on the 10th day. The lesions were typical.

Experiment 4.—To 100 cc. of nasal washings from two normal adults was added 1 cc. of a Berkefeld filtrate of mixed virus, 0.1 cc. of which produced paralysis in the control monkey in 7 days. 5 cc. of chemically pure ether were added to the mixture and the whole was shaken for 20 hours at room temperature. The ether was allowed to evaporate, and 2 cc. of the mixture were injected intracerebrally and 98 cc. intraperitoneally under ether anesthesia into a *Macacus rhesus*. The monkey remained well.

Berkefeld filters withhold even very minute particles in greater amount when they are contained within a viscid or glutinous liquid. All the washings contain mucus; hence a procedure was adopted to modify the mucin so as to avoid this difficulty without at the same time injuring unduly the virus itself. The procedure consists in treating the washings with sodium bicarbonate, filtering, and then concentrating *in vacuo* at 37°C. The virus is little injured. When 0.1 cc. of a filtrate, which is on the limits of a minimum lethal dose, is used, the resulting concentrated fluid may be ineffective; when 0.2 to 0.3 cc. is employed infection results. The next protocol in which 0.3 cc. of filtrate was used is an example of the method, but identical effects were obtained with 0.2 cc.

Experiment 5.—The nasal cavity of a normal adult was thoroughly syringed with 50 cc. of sterile distilled water. 0.3 cc. of a Berkefeld filtrate of virus was added, and, after thorough mixing, 0.25 gm. of dry sodium bicarbonate was added and the fluid shaken for 20 minutes with beads. After centrifugation at high speed for 3 minutes the fluid was decanted and passed through a Berkefeld candle V. The precipitate was washed and filtered through the same candle. The mixture of the filtrates was reduced *in vacuo* at 36°C. to a volume of 2 cc., which with rinsing water was transferred to a collodion sac and dialyzed for 1 hour.

Under ether anesthesia a *Macacus rhesus* received half (3.5 cc.) of the resulting liquid into the left, and the other half into the right cerebral hemisphere. No symptoms were observed until the 6th day when the monkey became ataxic and excitable. The monkey was prostrate on the 8th day and died on the 14th day after injection. Typical microscopic lesions of poliomyelitis were present.

The treatment of the washings with sodium bicarbonate renders the effect of the inoculation certain when the use of larger quantities of the virus with washings alone fails to confer infection. The probable cause of the discrepancy has become apparent only after a more minute study of the properties of the nasal washings; but that the sodium bicarbonate acts either by allowing more virus to pass through the filter or by removing certain inhibitory influences exerted by the washings is directly indicated.

Inactivating Effects of Nasal Secretions upon the Virus.

The results of the preceding experiments, which contained obvious discrepancies, suggested a closer study of the secretions of the nose and pharynx from the standpoint of a possible inhibiting or neutralizing action on the virus of poliomyelitis. For this purpose a variety of persons was studied; some were suffering from acute poliomyelitis, and the others were apparently normal individuals.

The nasopharynx was rinsed with double distilled water and the washings were fractionally sterilized by heating to 60°C. for 3 successive days. Each person's specimen was handled separately. In earlier experiments, in order to economize animals, the washings of several persons were often mixed. It now seems not improbable that discordant results follow this procedure. The virus employed was obtained by filtering a 5 per cent suspension of glycerolated poliomyelitic monkey spinal cord. To each 30 cc. of the washing 7.5 cc. of the filtered virus were added. The mixture was then incubated at

37°C. for 24 hours. Control mixtures of virus and distilled water were subjected to the same incubation. Each cubic centimeter of the mixtures then contained 0.2 cc. of the filtrate, or at least two minimum lethal doses of the virus. The results of the first tests are given in Table I.

TABLE I.

Inactivating Effects of Nasal Secretions upon the Virus.

Date.	Virus No.	Dose of virus filtrate.	In contact with nasal washings from.	Method of sterilizing nasal washings before addition of virus.	Temperature at which virus plus nasal washings were incubated for 24 hrs.	Result.
1916		*cc.*			°C.	
Feb. 16	32	0.2	Baby C.; age 3 yrs. Acute stage of poliomyelitis.	Heated 1 hr. at 60°C. on 3 successive days.	37	Monkey died. Typical poliomyelitic lesions.
Mar. 11	32	0.2	W. T., normal adult; age 39 yrs.	"	37	Monkey remained well.
June 2	48	0.2	C. A. R. and L. M. McK. (mixed), normal adults.	"	37	"
" 2	48	0.2	"	"	4	"
" 2	48	0.2	H. E. G., normal adult.	"	37	"

The results of this experiment suggest that the nasal washings of a person suffering from acute poliomyelitis may exercise no restraining influence upon an active virus, while those from healthy persons, under identical conditions of preparation, inhibit its activity.

The next experiment comprised tests on the nasal washings of eight apparently healthy persons. The results are recorded in Table II. At first sight it appears that of the eight specimens of washings, six possessed inhibiting properties and two did not. The question arose as to whether examination by a rhinologist, who would be unaware of the experiment, would disclose any differences in the nasal mucous membranes. These examinations, consented to by the

TABLE II.

Inactivating Effects of Nasal Secretions of Adults.

Date.	Dose of Berkefeld filtrate of Virus 48.	In contact with nasal washings from.	Method of sterilizing nasal washings before addition of virus.	Temperature at which virus plus nasal washings were incubated for 24 hrs.	Result.
1916	*cc.*			°C.	
Apr. 26	0.2	M. J. P., normal adult.	Heated 1 hr. at 60°C. on 3 successive days.	37	Monkey remained well.
" 26	0.2	E. S. S., " "	"	37	"
" 26	0.2	V. H. S., " "	"	37	"
" 26	0.2	C. A. R., " "	"	37	"
" 26	0.2	L. M. McK., normal adult.	"	37	"
" 26	0.2	G. H., normal adult.	"	37	"
" 26	0.2	J. P. B., " "	"	37	Monkey died. Typical lesions.
" 26	0.2	H. E. G., " "	"	37	"
" 26	0.2	Control (sterile water).		37	"
" 26	0.2	" (isotonic salt solution).		37	Monkey remained well.

persons, were kindly undertaken by Dr. M. C. Twitchell. His report is summarized in Table III. The only comment which the examination calls for is that while the anatomical condition of the nasal and adjacent mucosas in the six persons whose secretions contained inhibiting, inactivating, or neutralizing substances were normal, those of the other two were more or less pathologic. Just what the relation of this fact is to the effects of the secretions on the virus can only be surmised; but the test demonstrates that the secretions may frequently inhibit the action of the virus in monkeys. The control tests (Table II) show that, under the conditions of the experiments, distilled water injures the filtered virus less quickly than isotonic salt solution, a fact possibly dependent upon the different osmotic conditions present in the two fluids. The inactivation of

TABLE III.

Results of Rhinoscopy of the Subjects Recorded in Table II.

Case No.	Age.	Report.
	yrs.	
1 (M. J. P.)	23	Normal nasal respiration; no discharge; no history of colds. Mild hypertrophic rhinitis. Septum deflected slightly to left with large horizontal ridge.
2 (E. S. S.)	24	Normal nasal respiration; no discharge; no throat symptoms; no history of colds. Mild hypertrophic rhinitis; vocal bands red (subacute laryngitis).
3 (V. H. S.)	24	Normal nasal respiration; no discharge; no throat symptoms; no history of colds. Mild hypertrophic rhinitis. Two spurs on left side of septum.
4 (C. A. R.)	20	Normal nasal respiration; no discharge; no throat symptoms; no history of colds. Mild hypertrophic rhinitis; septum deflected, half closes right nasal cavity.
5 (L. M. McK.)	22	Normal nasal respiration; no discharge; no throat symptoms; no history of colds. Mild hypertrophic rhinitis; small ulcer on left side of septum: horizontal ridge on right side of septum.
(G. H.)	21	Normal nasal respiration; no discharge; no throat symptoms; no history of colds. Mild hypertrophic rhinitis. Acute pharyngitis of 3 days' duration.
7 (J. P. B.)*	22	Normal nasal respiration but easily obstructed when patient has a cold, especially left side of nose; secretion drops into throat on arising in the morning. Nose narrow; moderate hypertrophic rhinitis; large spur on right side of septum touches turbinate; small ulcer on left side of septum.
8 (H. E. G.)*	25	Nasal respiration interfered with, especially on right side; secretion drops into throat; frequent colds; has cold now. Septum deflected slightly to right; hypertrophic rhinitis; secretions found in right nasal cavity by anterior rhinoscopy. Acute pharyngitis and rhinitis.

* Remarks by Dr. Twitchell: "No. 7 shows the most marked chronic nasal trouble of all, and I should class it as moderate rather than severe. No. 8, at the time of examination, had an acute rhinitis and an acute pharyngitis. This to a certain extent obscures the findings in this case. Frequent colds are a marked feature in the history of chronic rhinitis. No. 8 is the only one giving this history. I should conclude that if a chronic rhinitis produces changes in the nasal secretions, No. 8 would be the one whose nasal secretions were the most changed."

the virus through dilution by the washings and incubation at 37°C. would appear to be excluded by the results of the tests with the secretions and with the controls.

Fluctuations in Inactivating Properties.

Attempts were made to ascertain whether the action described is a constant or a variable property of the secretions. For this purpose washings were made at different times, sterilized by discontinuous heating at 60°C., and tested against 0.2 cc. of the filtrate which in control tests was determined to be potent. The results of these tests are given in Table IV.

TABLE IV.

Fluctuations in Inactivating Properties.

Date.	Case.	Condition.	Dose of virus filtrate.	Result.
1916			*cc.*	
Mar. 11	W. T.	Apparently normal.	0.2	Neutralized.
June 16	"	" "	0.5	"
July 12	"	" "	0.2	Failed to neutralize.
Nov. 14	"	" "	0.2	Neutralized.
Apr. 26	H. E. G.	Chronic rhinitis.	0.2	Failed to neutralize.
June 2	"	" " (improved).	0.2	Neutralized.
July 12	"	Apparently normal.	0.2	Failed to neutralize.
Apr. 26	C. A. R.	" "	0.2	Neutralized.
June 2	"	" "	0.2	"
Apr. 26	G. H.	" "	0.2	"
Dec. 18	"	" "	0.2	"
Apr. 26	L. M. McK.	" "	0.2	"
June 2	"	" "	0.2	"
Apr. 26	E. S. S.	" "	0.2	"
July 12	"	Acute coryza.	0.2	Failed to neutralize.

Of four tests with the secretions of W. T., three neutralized the virus; of three with those of H. E. G., only one neutralized it; of two with washings from C. A. R., G. H., and L. M. McK., respectively, all neutralized it, while in the case of E. S. S., one neutralized and the other did not. The animals that did not come down were subsequently determined to be susceptible to inoculation with the virus, so that the neutralization effects could not have been simulated by an excessive resistance on their part.

In addition to the tests described, which were conducted chiefly with adults, several were made with washings from children either healthy or suffering from poliomyelitis. The results are not wholly concordant. A larger series may possibly clear up the discrepancies.

Aug. 9, 1916. The washings of C. A., an apparently healthy boy, age 14, failed to neutralize 0.2 cc. of filtrate.

Oct. 23, 1916. The washings of R. J., age 8, taken during the acute attack of poliomyelitis, but after immune serum had been administered, neutralized 0.2 cc. of filtrate. A control monkey developed fatal, typical poliomyelitis.

Nov. 14, 1916. The washings of R. C., age 8, taken on the 15th day of the attack of poliomyelitis neutralized 0.2 cc. of filtrate. This patient had not been treated with immune serum. The control animal developed typical fatal poliomyelitis.

Feb. 16, 1916. The washings of B. C., age 3, taken during the acute stage of poliomyelitis did not neutralize the filtrate. Immune serum had not been given.

While the number of observations is too small to draw definite conclusions, it is obvious that the secretions of apparently normal persons vary in the so called neutralizing power. Of the two patients with poliomyelitis whose secretions inhibited action of the filtrate, one had received immune serum, while the washings were taken from the other on the 15th day, or at a time when immunity principles are known to be present in the blood.[7] The third child with poliomyelitis yielded washings without neutralizing effect; but they were taken earlier (4th day) in the course of the infection and at a time when the immunity bodies were probably not yet abundantly present. It is possible that some relation exists between the presence of definite immunity principles in the circulating blood and the power of the nasal washings to neutralize the virus.

In each series of experiments the potency of the virus was established by control experiments, and subsequently all the monkeys not showing symptoms were tested for immunity by appropriate injections of the virus and were all found to have been susceptible to infection. Hence the lack of response was not caused by an immunity of the animals employed. The secretions of three persons out of six examined varied in their power to neutralize 0.2 cc. of the virus filtrate at different times under nearly identical conditions,

[7] Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1917, xxv, 499.

yet the only known clinical differences consisted in the presence of a rhinitis which appears to remove the inactivating power of the secretions.

Fluctuations of the Inactivating Power in Abnormal Nasal Conditions.

In April, 1916, the nasal secretions of E. S. S. neutralized 0.2 cc. of the virus filtrate, but 3 months later, during an attack of acute rhinitis, they did not. The washings from C. A. R. twice neutralized the same amount of virus at different times. Later, immediately following an acute rhinitis, no neutralizing power was observed, but the neutralizing power returned in 4 days.

The washings from H. E. G., taken when rhinoscopy revealed acute congestion of the nasal mucosa, did not possess neutralizing power, but 5 weeks later when the nasal condition had improved the washings showed the inactivating power. 6 weeks after the second test when there were no subjective symptoms of rhinitis, the washings failed to neutralize the virus. Finally, it will be recalled (Table III) that out of eight samples of nasal washings taken from apparently normal adults, only the two which were taken from subjects in which rhinoscopy revealed an acute rhinitis failed to inactivate the virus. H. E. G. is included in this list.

Effect of Fractional Sterilization and Filtration on Inactivation.

The experiments recorded indicate that the washings sterilized fractionally or passed through Berkefeld filters inactivate or neutralize virus mixed with them in the form of a filtrate of a suspension of the spinal cord of a poliomyelitic monkey. There can, therefore, be no doubt that the procedures do not themselves remove the neutralizing substances. Tests were then made to determine the comparative or quantitative effects of the procedures.

The quantity of filtrate employed for inoculation in this series of experiments was 0.4 cc., or more than four minimum lethal doses. The rinsings of the nasopharynx were made with redistilled water and they were reduced to a uniform volume of 15 cc. by concentration *in vacuo*. The fractional sterilization was carried out at 60°C. on 3 successive days. The washings and virus were left in contact 24

hours before the inoculations were made, in some instances at 37°C., in others at 4°C. The injections were intracerebral into *rhesus* monkeys under ether anesthesia. The results are given in Tables V and VI, and the comparison in Table VII.

The results lack absolute consistency. Considering the quantity of virus employed, the neutralizing action becomes more impressive. The variations in specimens from the same individual cannot now be accounted for. The existence of acute rhinitis, however, appears to diminish neutralizing power. Assuming that the process of neutralization is brought about by definite chemical bodies, they would seem to be thermolabile, since the neutralizing action of filtrates is definitely more pronounced than that of the heated specimens. Contact at 4°C. appears less effective in bringing about the neutralization than at 37°C. The prolongation of the incubation period noted in two instances is probably associated with partial but insufficient neutralization to reduce the virus below the minimum lethal dose.

Influence of Heat.

The results given above suggest that the inactivating influence is weakened or destroyed by heat. The following experiment gives more definite information concerning this fact.

Washings were taken on Nov. 16, 1916, from W. T., whose nasal secretions had on several occasions proved neutralizing. 60 cc. of sterile distilled water were used and the washings passed through a Berkefeld N candle.

To 10 cc. of washings filtrate were added 2.5 cc. of active virus filtrate and the mixture was incubated at 37°C. for 24 hours. 1 cc. of the mixture, representing 0.2 cc. of the virus filtrate, was injected intracerebrally, under ether anesthesia, into a *Macacus rhesus*. The monkey remained well.

35 cc. of the washings filtrate were reduced quickly *in vacuo* at a temperature between 60° and 70°C. to a volume of 5 cc. 1.25 cc. of active virus filtrate were added and the mixture was incubated at 37°C. for 24 hours. 1 cc. of the mixture, representing 0.2 cc. of virus filtrate, was injected intracerebrally, under ether anesthesia, into a *Macacus rhesus*. The monkey was completely paralyzed on the 7th day and died on the 8th day. Typical lesions of poliomyelitis were present.

The neutralizing substance is apparently rendered inactive by heating to 70°C., though this experiment does not exclude volatility as the reason for the disappearance of this substance. Other experiments, however, in which the concentrations were carried out *in vacuo* at 60°C. indicate that the neutralizing substances are not volatile.

TABLE V.

Neutralizing Power of Nasal Washings Heated to 60°C. for 1 Hour.

Monkey.	Dose of virus filtrate.	Fractionally sterilized nasal washings from.	Temperature at which virus plus nasal washings were incubated for 24 hrs.	Result.
	cc.		°C.	
A	0.4	W. T., normal adult.	37	Died in 33 days.
B	0.4	" " "	4	" " 31 "
C	0.4	G. H., " "	37	Remained well.
D	0.4	" " "	4	Died in 11 days.
E	0.4	C. A. R. (acute rhinitis).	37	" " 8 "
F	0.4	" " "	4	" " 8 "
G	0.4	Control (distilled water).	37	" " 14 "

TABLE VI.

Neutralizing Power of Nasal Washings Passed through a Berkefeld Filter.

Monkey.	Dose of filtrate of virus.	Berkefeld filtered nasal washings from.	Temperature at which virus plus nasal washings were incubated for 24 hrs.	Result.
	cc.		°C.	
H	0.4	W. T., normal adult.	37	Remained well.
I	0.4	" " "	4	Died in 16 days.
J	0.4	G. H., " "	37	" " 19 "
K	0.4	" " "	4	Remained well.
L	0.4	C. A. R. (4 days after acute rhinitis).	37	" "
M	0.4	" (4 " " " " ").	4	" "

TABLE VII.

Effect of Berkefeld Filtration and Heat on the Neutralizing Power of Nasal Washings

Nasal washings from.	Condition of person from whom washings were obtained.	Result of neutralizing test against 0.4 cc. of Berkefeld filtrate of virus.			
		Filtered (Berkefeld) washings plus virus allowed to remain for 24 hrs.		Fractionally sterilized washings (60° C. on 3 days) plus virus allowed to remain for 24 hrs.	
		37°C.	4°C.	37°C.	4°C.
W. T., adult.	Normal.	+*	−	±†	±†
C. A. R., adult.	Acute rhinitis.			−	−
" "	4 days after acute rhinitis.	+	+		
G. H., "	Normal.	−	+	+	−
Control (distilled water).				−	

* The sign + indicates neutralization; ±, marked prolongation of the incubation period preceding paralysis.

† Incubation period greatly prolonged. Monkeys developed no symptoms until 33 and 31 days, respectively, after inoculation.

DISCUSSION.

The power of the secretions of the nasopharynx of certain but not all individuals to bring about the inactivation or neutralization of the active virus of poliomyelitis has been demonstrated. The term active is employed to indicate that the samples of virus were obtained from strains adapted to the monkey, and could be relied upon to cause infection in the doses employed, almost without exception.

The inactivating property of the secretions mentioned is the more surprising in view of the resistance displayed by the poliomyelitic virus to such chemical antiseptics as glycerol and phenol.

In their manner of action, the neutralizing substances resemble more the specific immunity bodies contained within the blood serum of persons and monkeys who have suffered an attack of poliomyelitis. Like them, they appear to be thermolabile. And yet the experiments here recorded do not actually identify the two classes of substances.

It is known that the blood serum of certain adults who apparently

have never suffered from poliomyelitis is capable of neutralizing[8] the filtered poliomyelitic virus.[9] But in the few instances in which this property has been discovered, the adults yielding the serum had been in contact with acute cases of poliomyelitis, and artificial immunization cannot be excluded.

On the other hand, it seems not improbable that the inactivating or neutralizing power of the nasal secretions may play a part in protection against poliomyelitic infection, and even may represent an external system of defense against invasion of the virus by way of the nasopharyngeal mucosa.

If this view is supported by further studies, we should find that the secretions of children are less frequently neutralizing than those of adults, although many tests will be necessary to establish this distinction. In that case, we may find that the secretions of persons attacked by poliomyelitis at the period of onset of the disease lack neutralizing power, although later, when the immunization reactions have been aroused, inactivation may result, as has been shown to happen in particular instances in our series (page 516).

It appears, however, that the power of a given secretion to inactivate or neutralize the virus is not wholly a fixed one. Fluctuations in the property have been detected and described. Common and slight inflammatory conditions, *e.g.*, as in acute and even chronic rhinitis, apparently tend to remove or diminish the neutralizing power of the secretions. If this observation should be supported by further experiment, knowledge concerning one of the conditions favoring persistent contamination of the nasopharynx with the virus may be obtained. It does not follow, however, that this contamination need necessarily lead to infection, for the accomplishment of which disturbance of still other defensive mechanisms may be necessary. However, the production of healthy carriers of the poliomyelitic virus may rest upon the power or lack of power in the secretions to inactivate the virus. Should this be the case, then of many persons exposed only a fraction would become carriers, because the greater

[8] Flexner and Lewis, *J. Am. Med. Assn.*, 1910, liv, 1780.

[9] Peabody, F. W., Draper, G., and Dochez, A. R., A Clinical Study of Acute Poliomyelitis, Monograph of The Rockefeller Institute for Medical Research, No. 4, 1912.

part would possess secretions capable of neutralizing and hence destroying the virus.

The variation in inactivating power does not depend alone upon inflammatory changes. Irregularities have been noted which cannot now be explained. They may be merely apparent and depend upon the experimental method to which we are at present limited. Inoculation experiments in single series are not wholly trustworthy. Filtration through porcelain is also open to errors of experiment, since the blocking of the porous spaces may easily exclude essential constituents of the washings. Fractional sterilization is also not a wholly reliable means of preventing bacterial development and yet of retaining unimpaired labile organic constituents. In view of all this, some degree of irregularity is to be looked for.

If this property of the secretions to inactivate or neutralize the virus of poliomyelitis is established, comparative tests should be made on large groups of persons at different seasons of the year in order to determine whether it bears any relation to the seasonal prevalence of poliomyelitis. We are engaged now in collecting observations covering this point; but reference to the tables will show that most of the tests were made during the spring, summer, and autumn. Moreover, they embraced few children of the most susceptible ages.

SUMMARY.

1. The results of 56 experiments have shown that washings of the nasal and pharyngeal mucosas possess definite power to inactivate or neutralize the active virus of poliomyelitis.

2. This power is not absolutely fixed, but is subject to fluctuation in a given person. Apparently inflammatory conditions of the upper air passages tend to remove or diminish the power of neutralization. But irregularities have been noted, even in the absence of these conditions.

3. Too few tests have been made thus far to ascertain whether adults and children differ with respect to the existence of this neutralizing property in the nasal secretions. While the inactivating property was absent from the secretions of one child during the first days of poliomyelitis, it was present in another to whom immune

serum was administered, and in still another on the 15th day of illness when convalescence was established.

4. The neutralizing substance is water-soluble and appears not to be inorganic; it appears to be more or less thermolabile, and its action does not depend upon the presence of mucin as such.

5. It is suggested that the production of healthy carriers through contamination with the virus of poliomyelitis may be determined by the presence or absence of this inactivating or neutralizing property in the secretions. Whether this effect operates to prevent actual invasion of the virus and production of infection can only be conjectured. Probably the property is merely accessory and not the essential element on which defense against infection rests. It is more probable that other factors exist which help to determine the issue of the delicate adjustment between contamination and infection.

THE RELATION OF THE MENINGES AND CHOROID PLEXUS TO POLIOMYELITIC INFECTION.

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The prevention or promotion of an infectious disease is determined by the interaction of several factors. This may be assumed to be true of epidemic poliomyelitis. We know that the presence of the microorganism, or virus, of that disease upon the mucous membrane of the nose and throat does not necessarily lead to infection. This form of contamination apparently gives rise to a class of healthy carriers of the microbic cause of poliomyelitis. The prevailing view, based on analogy with other infectious diseases in which the portal of entry of the causative microorganism is the upper respiratory mucous membrane, notably epidemic meningitis, is to the effect that these carriers are several times as numerous as actual cases of infection. Obviously, therefore, mechanisms exist to protect the contaminated persons from the pathogenic action of the virus which they carry.

The incidence of poliomyelitis in communities in which it has prevailed has been low. Thus the case incidence of the Greater New York epidemic (1916) was 1.59 per 1,000 of the population.[1] From this fact a high degree of individual insusceptibility has been inferred,[2] and the basis of the condition has been sought in the notion that epidemic poliomyelitis is a far more common condition than has been supposed, and in very mild form may prevail extensively without being recognized as that disease.[3] Since even a slight attack leaves an enduring protection,[4] a state of general immunity has been created

[1] Emerson, H., *Am. J. Med. Sc.*, 1917, cliii, 160.

[2] Flexner, S., *J. Am. Med. Assn.*, 1916, lxvii, 279.

[3] Wernstedt, W., *Communications Inst. méd. État à Stockholm*, 1912, iii, 235.

[4] Flexner, S., and Lewis, P. A., *J. Am. Med. Assn.*, 1910, liv, 45; *J. Exp. Med.*, 1910, xii, 227.

which manifests itself later during severe epidemics attended with paralysis and high mortality. While our present knowledge does not permit a final statement as to the applicability of this hypothesis even to Northern European countries in which poliomyelitis has been endemic for the past 30 years, it seems entirely inapplicable to America and the other countries which have only recently come widely under the influence of the epidemic disease.

The discovery of the main portals by which the virus enters the body has focused attention on the conditions which favor or hinder its entrance.[5,6] Having reached the upper respiratory mucosa the virus may take either of two routes in invading the central nervous organs. It may penetrate first into the local blood vessels and be carried into the circulation and thence to the nervous tissues, or it may pass into the lymphatic vessels surrounding the olfactory nerves and ascend more directly to the brain, medulla, and spinal cord.

Experimental evidence suggests the latter route.[7] It is difficult to infect monkeys with highly active poliomyelitic virus by way of the blood; and, conversely, it is easier to infect them by way of the nasal mucosa.[6] It is the function of the choroid plexus and the pial lymphatic vessels to exclude the virus present in the blood from the nervous tissues. Once these protective structures are injured, the exclusion ceases and infection can be made to follow readily. Flexner and Amoss[8] found that the injection of normal horse serum into the meninges, through which a transient aseptic meningitis is produced, is an effective way of overcoming the obstacle interposed by the structures mentioned. Intactness or permeability may conceivably play a part in determining whether infection is to ensue or not. When the virus enters directly from the nasal mucosa to the brain, the medulla and lastly the spinal cord become infected,[7] from which it would appear that the virus entering the central nervous organs by way of

[5] Flexner and Lewis, *J. Am. Med. Assn.*, 1910, liv, 535.

[6] Levaditi, C., and Landsteiner, K., *Compt. rend. Soc. biol.*, 1910, lxviii, 417. Flexner and Lewis, *J. Am. Med. Assn.*, 1910, liv, 1140.

[7] Flexner, S., and Clark, P. F., *Proc. Soc. Exp. Biol. and Med.*, 1912–13, x, 1. Flexner, *Lancet*, 1912, ii, 1271; *Science*, 1912, xxxvi, 685; *Brit. Med. J.*, 1912, ii, 1261.

[8] Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1914, xx, 249.

the olfactory nerves permeates the organs continuously and is not distributed by the general circulation.

In discussing the defensive mechanisms, it is desirable in the first place to consider the ultimate fate of the virus which reaches the nasal mucosa as it does in many more persons than develop poliomyelitis. Amoss and Taylor[9] have found that the secretions of the mucous membrane are capable of neutralizing or inactivating the virus; and their experiments show also that this neutralizing property is absent altogether from the secretions of some persons and fluctuates in those of others, being present at one time and not at another. We may, indeed, view this destructive power as a means by which the number of persons becoming contaminated with the virus in the course of epidemics is diminished, as well as a mechanism through which infection may actually be prevented. Hence it may constitute one factor in the complex state to which the term susceptibility is applied. That still other factors enter into the perfection of this state is indicated by the experiments to be described.

Meningeal Disturbances and Intravenous Inoculation.

The routes through which infection with the poliomyelitic virus may be accomplished in monkeys have been closely studied. In order of ease and constancy they are: the intracerebral, intranasal, intraperitoneal, intraneural,[10] subcutaneous, and intravenous routes. The virus may also enter by other and extraordinary channels which have been little studied; namely, after intraocular injection[11, 8] and after feeding in animals narcotized with opium.[12] The intracerebral route gives not only the most constant results, but it exceeds the others in delicacy of response. This fact has been emphasized in a previous paper as has the power of the virus to be augmented after an intravenous inoculation through an aseptic meningitis induced by the intraspinal injection of normal horse serum.[8] Since then we have

[9] Amoss, H. L., and Taylor, E., *J. Exp. Med.*, 1917, xxv, 507.

[10] Some authors regard intrasciatic inoculation as next to intracerebral in effectiveness; in our experience it constitutes an uncertain portal of entry of the virus.

[11] Landsteiner and Levaditi, *Compt. rend. Soc. biol.*, 1909, lxvii, 787.

[12] Leiner, C., and von Wiesner, R., *Wien. klin. Woch.*, 1910, xxiii, 91.

ascertained that an intranasal application of the virus, otherwise ineffective, may be made infectious by a similar aseptic inflammation of the meninges. The meningeal mechanism, which includes the choroid plexus, has indeed proved to be not only determinative in respect to the effect of an inoculation of the virus, but also of remarkable delicacy of adjustment. Pathologic changes of almost incredably slight character may set aside its protective function.

Horse Serum.—The intraspinal injection of normal horse serum brings about in monkeys profound changes in the meningeal mechanism.[8] Besides producing a rich cellular, protein exudate, the epithelial coverings of the choroid plexus are rendered more permeable. Under these circumstances an otherwise ineffective quantity of the virus introduced into the circulation becomes infectious. But even so the quantity of virus effective by way of the circulation exceeds considerably that necessary to produce infection by intracerebral injection.

Experiment 1 (Control).—Dec. 13, 1916. A *Macacus rhesus* received an intravenous injection of 50 cc. of a centrifuged 5 per cent suspension of active monkey spinal cord and medulla,[13] 0.1 cc. of a Berkefeld filtrate of which was infectious by intracerebral inoculation. The monkey remained well.

Experiment 2.—Dec. 21, 1916. 4 p.m. 2 cc. of normal horse serum were injected intraspinally into a *Macacus rhesus.* Dec. 22, 11 a.m. 1 cc. of centrifuged virus as in Experiment 1 was injected intravenously. The monkey remained well.

Experiment 3.—The same as Experiment 2, with 5 cc. of the centrifuged virus. The monkey remained well.

Experiment 4.—The same as Experiments 2 and 3, with 10 cc. of the centrifuged virus. Paralysis on the 6th day and death on the 9th day following the intravenous injection.

The greater efficacy of the intracerebral route of inoculation is brought out by the experiments. The discrepancy is greater even than it appears to be because the filtration of the centrifuged suspension removes a large part of the protein content and with it, of course, much, and perhaps most of the virus.

In the past the intracerebral mode of inoculation has been relied

[13] In order to avoid immediate toxic effects, the emulsion must be made from nervous organs freshly removed from the paralyzed monkeys. Specimens kept at 4°C. or in 50 per cent glycerol are not satisfactory. Their injection is often followed in a short time by a drop in blood pressure and respiratory failure.

upon in therapeutic and disinfection tests with the poliomyelitic virus because it alone gave constant results. This was a disadvantage since, in the first place, the reaction of monkeys to an intracerebral inoculation of the adapted virus is far more severe than the response of human beings to the infection in the ordinary way. With the active virus employed in this manner, the mortality among the inoculated monkeys is approximately 100 per cent. In the next place the choice of disinfecting agents was necessarily limited to those which could be introduced into the cerebrum along with the virus. These disadvantages have been overcome through the promoting action of horse serum and other agents, some of which were hitherto regarded as indifferent, introduced into the meninges.

Normal Monkey Serum.—An homologous serum injected into the meninges sets up an aseptic meningitis, of lighter grade than that induced by a heterologous serum. Normal monkey serum was tested for its power to promote infection following an intravenous injection of the poliomyelitic virus.

Experiment 5.—Dec. 12, 1916. A *Macacus rhesus* received an intraspinal injection of 2 cc. of clear, hemoglobin-free normal monkey serum. Dec. 13. Intravenous injection of 50 cc of a centrifuged 5 per cent suspension of active spinal cord and medulla. Dec. 18. Animal moves somewhat stiffly; ataxia; tremor of head; ptosis; shoulder muscles weak. Dec. 19. Died.

The autopsy showed typical lesions of poliomyelitis.

As this experiment shows, normal monkey serum resembles normal horse serum in promoting infection from an intravenous injection of the poliomyelitic virus. Experiments 6, 7, 8, and 9 indicate that even a less degree of inflammatory reaction than that induced by normal serum suffices to open the way for the virus present in the blood to reach the nervous organs.

Salt, Ringer's, and Locke's Solutions.—Of the three fluids mentioned, those which physiologically are most indifferent are Ringer's and Locke's solutions. As far as visible responses are concerned, all produce less inflammation of the meninges than either horse serum or monkey serum. And yet they are clearly proved by the reactions to be foreign and in a degree injurious to the meningeal structures.

Experiment 6.—Dec. 19, 1916. 5 p.m. 2 cc. of sterile isotonic salt solution were injected intraspinally into a *Macacus rhesus*. Dec. 20, 11 a.m. 50 cc. of a centri-

fuged 5 per cent suspension of active spinal cord and medulla were injected intravenously. Dec. 26. Ataxia; tremor of head; double ptosis. Dec. 27. Died.

The autopsy showed typical and marked lesions of poliomyelitis.

Experiment 7.—Dec. 19, 1916. 5 p.m. 2 cc. of sterile Ringer's solution were injected intraspinally into a *Macacus rhesus*. Dec. 20, 11 a.m. 50 cc. of centrifuged fluid similar to that in Experiment 6 were injected intravenously. Dec. 29. Ataxia; tremor of head. Dec. 30. Shoulder and back muscles weak. Jan. 2, 1917. Paralysis extended. Died.

The autopsy showed typical lesions of poliomyelitis.

Experiment 8.—Macacus rhesus. This experiment was exactly like the preceding one, except that sterile Locke's solution was used. Jan. 5, 1917. Intraspinal injection of Locke's solution. Jan. 11. The first symptoms appeared. Jan. 15. Died.

The autopsy showed typical poliomyelitic lesions.

In the next experiment, Locke's solution plus 0.5 per cent gelatin were employed.[14]

Experiment 9.—Jan. 23, 1917. 4 p.m. 2 cc. of sterile Locke's solution containing 0.5 per cent gelatin were injected intraspinally into a *Macacus rhesus*. Jan. 24, 10 a.m. 50 cc. of a centrifuged 5 per cent suspension of spinal cord and medulla were injected intravenously. Feb. 2. Paralysis of legs; shoulders weak. Feb. 4. Prostrate. Feb. 5. Died.

The autopsy showed typical lesions of poliomyelitis.

It is obvious that even when the irritative and inflammatory changes induced in the meningeal structures are actually very slight, they suffice to remove the power of the intact organs to exclude the virus from the interstices of the central nervous tissues. These observations, in themselves important, unexpectedly confirm and extend an earlier experiment in which the injection of salt solution into a cerebral hemisphere promoted infection with a virus, otherwise ineffective by that route, introduced into the veins.[8]

Homologous and Autologous Cerebrospinal Fluid and Simple Lumbar Puncture.

Since it now appeared that very slight changes in the meningeal structures enabled the virus circulating in the blood to penetrate and

[14] Rous and Turner (*J. Exp. Med.*, 1916, xxiii, 219) observed that a fluid of that composition was an excellent medium for preserving red corpuscles intact for a considerable period and surpassed in that respect the other artificial physiologically balanced fluids tested.

multiply within the central nervous organs, the question arose as to the limit of the irritative process sufficing for this purpose. The next experiments related to (1) simple lumbar puncture, (2) the removal and return of the cerebrospinal fluid in the same monkey, and (3) the withdrawal of the fluid from one monkey and its replacement with the fluid taken from other monkeys.

Simple Lumbar Puncture.—By this term is meant the removal of fluid by lumbar puncture under conditions in which no blood whatever is drawn by the operation. If a trace of blood entered the needle, the animal was not regarded as suitable for the test.

Experiment 10.—Dec. 21, 1916. 4 p.m. 2 cc. of clear fluid were withdrawn from a *Macacus rhesus* by lumbar puncture. Dec. 22, 12 noon. Intravenous injection of 50 cc. of a centrifuged 5 per cent suspension of active spinal cord and medulla. No symptoms developed.

Withdrawal and Return of Autologous Fluid.—In this experiment, as in the preceding one, the merest tinge of blood in the withdrawn fluid renders the animal unsuitable.

Experiment 11.—Jan. 15, 1917. 4 p.m. 2 cc. of clear fluid were withdrawn from a *Macacus rhesus* by lumbar puncture. 5 p.m. The same fluid was returned by lumbar puncture. Jan. 16, 10.30 a.m. Intravenous injection of 50 cc. of a centrifuged 5 per cent suspension of active spinal cord and medulla. No symptoms developed.

Introduction of Homologous Fluid.—The same precautions were followed in this experiment to avoid the slightest hemorrhage in performing the lumbar puncture.

Experiment 12.—Jan. 5, 1917. 6 p.m. 2 cc. of clear cerebrospinal fluid were withdrawn from a *Macacus rhesus* by lumbar puncture and replaced by 2 cc. of clear fluid withdrawn 3 minutes earlier from a normal monkey of the same species. Jan. 6, 11 a.m. Intravenous injection of 50 cc. of a centrifuged 5 per cent suspension of active spinal cord and medulla. Jan. 14. Legs, arm, and back weak. Jan. 15. Prostrate. Feb. 2. Died.

The autopsy showed characteristic lesions of poliomyelitis.

Experiment 13.—Feb. 8, 1917. 4 p.m. 2 cc. of clear cerebrospinal fluid removed 30 minutes before from two normal *Macacus rhesus* monkeys were injected intraspinally into a monkey of the same species. Feb. 9, 10 a.m. Intravenous injection of 50 cc. of a centrifuged 5 per cent suspension of active spinal cord and medulla. Feb. 14. Paralysis of legs. Feb. 15. Prostrate.

The autopsy showed typical lesions of poliomyelitis.

This experiment has not succeeded in every instance.

That the production of even slight hemorrhage in withdrawing the cerebrospinal fluid may seriously alter the result is shown by the next experiment.

Experiment 14.—Feb. 15, 1917. 4 p.m. 1 cc. of cerebrospinal fluid slightly tinged with blood was withdrawn from a *Macacus rhesus* by lumbar puncture. The quantity of blood present in the fluid, estimated on the basis of red corpuscles present, was perhaps one-fiftieth of the total volume of the fluid. Feb. 16, 10 a.m. Intravenous injection of 50 cc. of a centrifuged 5 per cent suspension of active virus. Feb. 20, a.m. Ataxia; tonic convulsions. p.m. Prostrate. Feb. 21. Died.

The autopsy showed typical lesions of poliomyelitis.

The preceding experiments indicate that (1) simple uncomplicated lumbar puncture does not lead to infection after an intravenous inoculation; (2) the simple removal and return of the same cerebrospinal fluid in a given animal is likewise without effect; while (3) the interchange of fluid from one animal to another does lead to infection, although not in every instance. Moreover, injury to the blood vessels of the meninges also promotes the infection. The infection in the last example may be due either to the escape directly of the inoculated virus from the blood through the injured vessels into the meninges, or what is more probable, to the blood's entering the subarachnoid space and setting up a mild inflammatory reaction, which, as we have seen, suffices to promote the infection. Sometimes the interchange of fluids fails to be followed by an infection. This failure may be due to one of several causes: the two fluids interchanged may be essentially so alike in composition as to constitute practical identity; the test then becomes equivalent to the autologous experiment; or the meningeal structures in certain monkeys may be less responsive to such a mild irritant and resist its action altogether.

It is more remarkable that the irritative response should occur than that it should in some instances fail to take place. The experiments emphasize the extraordinary sensitiveness of the meningeal protective adjustment through which exclusion of the poliomyelitic virus, and doubtless other injurious substances, from the interior of the nervous organs, is accomplished. They suggest that if alterations as slight as these suffice to produce infection, the essential meningeal mechanism may have to be regarded as in no small part determinative of the development of poliomyelitic disease.

Serum Promotion of Intranasal and Subcutaneous Infection.

Following Flexner and Lewis's[5] demonstration that the virus of poliomyelitis introduced into the cerebrum escapes by way of the nasal mucosa, Levaditi and Landsteiner[6] first determined that the application of the virus to the nasal mucosa would lead to infection. As already stated, the intranasal method is a less constant means of producing infection than the intracerebral and requires somewhat greater quantities of the virus.

In the following experiments inoculation was accomplished by packing the left naris with a plug of absorbent cotton saturated with crushed spinal cord of a recently paralyzed monkey. The plug was left in position for 2 hours and then removed, and not infrequently it showed slight tingeing with blood.

We have found that packing for 2 hours is insufficient to lead to infection in normal monkeys. But when monkeys have previously received an intraspinal injection of horse serum, infection results.

Experiment 15.—Jan. 2, 1917. 5 p.m. 2 cc. of normal horse serum were injected intraspinally into a *Macacus rhesus*. Jan. 3, 11 a.m. A pledget of cotton soaked with fresh poliomyelitic spinal cord of a monkey was packed in the left naris. When removed 2 hours later it was slightly stained with blood. Jan. 9. Protects right arm. Jan. 11. Tremor of head; ataxia; considerable spasticity of legs and weakness of deltoids. Cerebrospinal fluid contains 2,680 cells per c.mm. and increased globulin. Jan. 12. Prostrate. Jan. 14. Died.

The autopsy showed characteristic lesions of poliomyelitis.

Experiment 16.—Feb. 8, 1917. 4 p.m. A *Macacus rhesus* received an intraspinal injection of 2 cc. of normal horse serum. Feb. 9, 10.30 a.m. A nasal plug saturated with poliomyelitic spinal cord and medulla was introduced and left in the left naris for 2 hours. Feb. 18. Paralysis in left arm; weakness of back. Feb. 19. Prostrate. Feb. 20. Etherized.

The autopsy showed typical lesions of poliomyelitis.

The same method of promoting infection was used in the instances of intrasciatic and of subcutaneous inoculation. In two trials no result was accomplished in the former; while in the latter infection took place.

Experiment 17.—Jan. 9, 1917. 5 p.m. 2 cc. of normal horse serum were injected intraspinally into a *Macacus rhesus*. Jan. 10, 2 p.m. Subcutaneous injection of 1 cc. of a 5 per cent suspension of spinal cord and medulla of a poliomye-

litic monkey. Jan. 20. Arms paralyzed; back weak; facial asymmetry. Jan. 21. Died.

The autopsy showed typical lesions of poliomyelitis.

A control animal in which no horse serum had been inoculated did not respond to the subcutaneous inoculation alone.

This series of experiments indicates that preexisting pathologic conditions in the meningeal structures promote infection in poliomyelitis not only when the virus is present in the blood, but also when it reaches the central nervous organs by way of the nerves, as in the case of intranasal inoculation and probably also of subcutaneous inoculation. The promoting influence of the serum in intranasal inoculation acquires special significance in view of the fact that the nasal mucosa is the usual portal of entry of the virus into the nervous organs in human beings;[15] that is, the intact meninges are a protection from nasal as from intravenous inoculation with the virus of poliomyelitis, as, conversely, the pathologically altered meninges are a cause of heightened susceptibility to the action of the virus present in either place.

Immune Serum and Heightened Susceptibility.

The experiments described show that not only normal horse and monkey serum, but fluids of far less irritative nature promote, when introduced into the meninges, infection with the virus of poliomyelitis. Earlier experiments, on the other hand, have shown that infection with the virus may be prevented by an immune serum even when the inoculation is made into the cerebrum.[16] Would infection therefore be prevented if an immune serum was injected into the meninges previous to the intravenous administration of the virus? For if infection does not occur, the difference would be attributable only to the presence within the immune serum of the neutralizing substances for the virus.

Experiment 18.—Dec. 21, 1916. 5 p.m. 2 cc. of immune monkey serum pooled from three animals which had recovered from an attack of experimental poliomyelitis were injected intraspinally into a *Macacus rhesus*. Dec. 22, 11 a.m. 50 cc. of a centrifuged 5 per cent suspension of active spinal cord and medulla of a paralyzed monkey were injected intravenously. No symptoms appeared.

[15] Flexner, S., *J. Am. Med. Assn.*, 1910, lv, 1105.

[16] Flexner and Lewis, *J. Am. Med. Assn.*, 1910, liv, 1780; lv, 662.

This experiment is conclusive in that it indicates that an immune serum alone of all the irritative fluids injected into the meninges prevents the infection. The experiment has a bearing also on the serum therapy of human poliomyelitis and upon the question of the employment for intraspinal injection of normal sera and other fluids of more or less irritative character.

The next series of experiments relates to the protecting power of an immune serum when it is employed to overcome the effect on the infection of an intraspinal injection, either of normal monkey serum or of normal salt solution. That protection would thus be afforded by the immune serum was regarded as certain, in view of the power which it had already displayed to hold up an infection by means of the intravenous administration of the centrifuged virus following the intraspinal injection of horse serum.[8]

Experiment 19.—Dec. 19, 1916. 4.30 p.m. 2 cc. of normal monkey serum were injected intraspinally into a *Macacus rhesus*. Dec. 20, 1 p.m. 50 cc. of a centrifuged 5 per cent suspension of active spinal cord and medulla were injected intravenously. 1.30 p.m. Intraspinal injection of 3 cc. of pooled immune monkey serum. Dec. 26. Ataxia; head tremor; ptosis. Dec. 27. Symptoms more marked. Dec. 28. Widespread weakness of muscles. Dec. 30. Prostrate. This animal slowly recovered with marked residual paralysis.

Experiment 20.—Dec. 19, 1916. 2 cc. of normal monkey serum were injected intraspinally into a *Macacus rhesus*. Dec. 20, 12 noon. 50 cc. of a centrifuged 5 per cent suspension similar to that used in the previous experiment were injected intravenously. 12.20 p.m. 3 cc. of pooled immune serum were injected intraspinally. The immune serum was given on the next 3 successive days, and after a 2 days' rest on 3 more successive days. No symptoms developed.

Experiment 21.—This experiment was an exact repetition of Experiment 20, and yielded the same results.

The inflammatory changes induced by normal monkey serum similar to those brought about by normal horse serum require, apparently, more than a single injection of the immune serum to prevent infection after intravenous injection. Two or three injections at 24 hour intervals would suffice, for within that period the defect would have probably been healed and the major part of the virus neutralized. Moreover, it has been shown[17] that large doses of the virus injected into the blood begin to disappear in 96 hours and the virus

[17] Flexner and Amoss, *J. Exp. Med.*, 1914, xix, 411.

no longer circulates in detectable quantities after 120 hours. After inflammation has been produced in the meninges by normal monkey serum a single intraspinal injection of the immune serum mitigated the severity of the infection, since, contrary to the rule, the paralyzed animal lived.

The last experiment of this series was made with normal salt solution. It repeats exactly the one in which normal monkey serum was injected intraspinally, the centrifuged virus intravenously, and one dose of immune serum intraspinally. In conformity with the smaller damage inflicted upon the meningeal structures, a single dose of the immune serum is observed to prevent infection.

Experiment 22.—Jan. 15, 1917. 3.30 p.m. 2 cc. of normal salt solution were injected intraspinally into a *Macacus rhesus*. Jan. 16, 10 a.m. 50 cc. of a centrifuged 5 per cent suspension of active spinal cord and medulla were injected intravenously. 10.20 a.m. Intraspinal injection of 3 cc. of pooled immune serum. No symptoms developed.

This group of experiments confirms and extends somewhat the earlier ones made with normal horse serum. It now appears not only that an immune serum injected intraspinally is protective, but that the degree of its efficiency is more or less proportional to the injury which the meningeal structures have suffered from the irritating substances employed to increase their permeability. The slighter the inflammation, the more readily and quickly the injury is repaired; with isotonic salt solution a single dose of the immune serum suffices to prevent infection, while with monkey and horse serum several doses are required.

CONCLUSIONS.

Among the mechanisms which defend the body from infection with the virus of poliomyelitis is the meningeal-choroid plexus complex, which normally is capable of excluding the circulating virus from the central nervous organs. The complex plays a part also in preventing infection from virus present upon the nasal mucosa.

Aseptic fluids which irritate, inflame, or even slightly alter the integrity of the meninges and choroid plexus diminish or remove their protective function.

Normal monkey or horse serum, isotonic salt solution, and Ringer's

and Locke's solutions, when injected into the meninges, promote infection with the virus of poliomyelitis introduced into the blood, the nose, or the subcutaneous tissues.

Simple lumbar puncture and the withdrawal and return of the cerebrospinal fluid in normal monkeys, hemorrhage having been absolutely avoided, do not promote infection with virus injected into the blood; while the replacement of the cerebrospinal fluid of one monkey with that of another does in some instances lead to infection. Simple lumbar puncture attended with even very slight hemorrhage opens the way for the passage of the virus from the blood into the central nervous tissues, and thus promotes infection.

Hence, changes in the structure or function of the meningeal-choroid plexus complex, too slight to be detected by chemical and cellular changes in the cerebrospinal fluid or by morphological alterations, suffice to diminish in an essential manner its protective powers.

Of all the irritant fluids tested, immune serum alone injected into the meninges is not succeeded by infection from the virus introduced into the blood.

The protective property of the immune serum is capable of overcoming the promoting action of normal monkey and horse serum and the other irritants mentioned.

The importance first of the meningeal-choroid plexus complex in preventing infection with the virus of poliomyelitis, and next of immune serum in offsetting the disadvantages and dangers arising from defects in the mechanism is apparent, as is the bearing of the experiments reported on the serum therapy of epidemic poliomyelitis.

SURVIVAL OF THE POLIOMYELITIC VIRUS FOR SIX YEARS IN GLYCEROL.

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Plates 40 to 43.

(Received for publication, February 6, 1917.)

The reappearance of poliomyelitis in severe form in the United States during the summer and autumn of 1916 has revived the question of the conditions under which the microbic cause, or virus, of the disease survives outside the human body. The problem of the source of the epidemic is still unsolved: whether, for example, the virus was newly imported or merely a survival from previous epidemics of the disease. We are not now in a position to answer this question. Our present knowledge indicates that the virus does not multiply in nature outside the human body, but it is possible that it may survive upon human carriers or elsewhere without actually multiplying.

The question of the power of the virus to resist extraneous influences has been sharpened by recent publications connecting the streptococcus with the etiology of poliomyelitis. Experience has shown that streptococci in animal tissues, for example, do not retain viability over long periods of time. We ascertained early in the experimental investigation of poliomyelitis that the virus in the central nervous tissues resists glycerolation,[1] and later we reported instances of survival after 25 months' immersion in glycerol.[2] In the course of investigations on poliomyelitis conducted at The Rockefeller Institute, specimens of nervous tissues from human beings and monkeys, the subjects of poliomyelitis, have been regularly put aside in 50 per cent glycerol in the refrigerator, the temperature of which has been

[1] Flexner, S., and Lewis, P. A., *J. Am. Med. Assn.*, 1910, liv, 45.

[2] Flexner, S., Clark, P. F., and Amoss, H. L., *J. Exp. Med.*, 1914, xix, 205.

kept at approximately 4°C. Some of the tissues were set aside in 1910, and others later. We have recently tested certain specimens for activity and ascertained that monkeys may be infected with samples 6 and 4 years old.

The spinal cord and medulla were cut into small cubes which were immersed in a large excess of 50 per cent glycerol previously sterilized in the autoclave. When portions were removed for purposes of inoculation, tests were made for bacteria by imbedding fragments of the tissue in deep tubes of glucose-ascitic-fluid-agar and inoculating the tissue on the surface of tubes of that medium. The tubes were incubated for several days. On microscopic examination no growth was discovered.

Experiment 1.—Human spinal cord and medulla kept in glycerol since August, 1911, and September, 1912. Fragments from the two specimens were combined for inoculation. Hence the more recent sample of tissue had been put aside more than 4 years before. Dec. 7, 1916. A *Macacus rhesus* was given an intracerebral injection, under ether anesthesia, of a heavy suspension of the tissues. Dec. 11. 3 cc. injected into the peritoneal cavity. Jan. 4, 1917. No symptoms. A fresh suspension was prepared of the same tissues, of which 3 cc. were given intracerebrally and 5 cc. intraperitoneally. Jan. 17. Excitement; ataxia; tremor of head; facial asymmetry; weakness of right deltoid. Jan. 22. The symptoms have advanced slightly. Jan. 27. Injected 2 cc. of suspension intracerebrally and 5 cc. intraperitoneally. Jan. 31. Symptoms slowly increasing. Etherized for histological examination and for purposes of reinoculation.

Histology.—Lesions occur in the medulla, spinal cord, and intervertebral ganglia. Those present in the medulla consist (1) of lymphocytic infiltration of the vessels in the meninges and (2) of slight perivascular infiltration of the superficial internal vessels. There is no general lymphocytic infiltration and no necrosis of nerve cells. The lesions in the spinal cord are focal. The cervical cord shows diffuse lymphocytic infiltration, necrosis of anterior horn nerve cells, and a small degree of neurophagocytosis. The blood vessels of the gray matter are moderately infiltrated with mononuclear cells. The lumbar cord is the seat of marked perivascular infiltration affecting chiefly the gray matter, but the meningeal vessels are also heavily surrounded (Fig. 1). The anterior gray matter is diffusely, but not severely, infiltrated with lymphocytic cells and no neurophagocytosis is present in the sections studied. The intervertebral ganglia show slight infiltration with lymphocytes and no nerve cell degeneration.

Experiment 2.—Monkey spinal cord and medulla. Several specimens of tissue from monkeys inoculated with M. A. virus in the spring and autumn of 1910 were combined for testing. They had been kept in 50 per cent glycerol for 6 years or longer at 4°C. Culture tests, similar to those described in Experi-

ment 1, were negative. Dec. 9, 1916. A *Macacus rhesus* received, under ether anesthesia, an intracerebral inoculation of 3 cc. of a heavy suspension. Dec. 12. 13 cc. of the same suspension were injected into the peritoneal cavity. Jan. 4, 1917. No symptoms had appeared. Injected 2 cc. of a fresh suspension made from the same tissues intracerebrally and 7 cc. intraperitoneally. Jan. 13. Ataxia; shoulder and leg muscles weak. Jan. 14. Weakness advancing; animal unable to climb. Jan. 17. Prostrate. Etherized.

Histology.—Sections prepared from the medulla, spinal cord, and intervertebral ganglia were studied. The medulla shows marked perivascular lymphocytic infiltrative lesions of the meninges and less of the nervous tissue itself. But within the latter are small focal lymphocytic accumulations sometimes about degenerating nerve cells. The spinal cord shows more pronounced lesions, especially of the anterior gray matter. Diffuse lymphocytic and edematous infiltration as well as marked perivascular cellular infiltrations are prominent (Fig. 2). The blood vessels of the white matter are widely affected. Hyaline degeneration of motor nerve cells is frequent, but little neurophagocytosis is going on (Fig. 3). The intervertebral ganglia show typical focal infiltrative lymphocytic lesions, but no necrosis of ganglion cells (Fig. 4).

The lesions in the nervous organs of the second monkey exceed in extent and severity those of the first. This is in conformity with the greater pathogenicity displayed for monkeys of the virus which has become adapted to that species.

The experiments described show that the virus of epidemic poliomyelitis as contained in the central nervous organs of human beings, the subjects of epidemic poliomyelitis, and of monkeys in which the experimental disease has been induced, survives for many years in the weak disinfectant glycerol. The manner in which the reinoculations which led to paralysis and to characteristic histological lesions were made indicates that during the periods mentioned, the virus suffered reduction in activity either because of mere diminution in the number of microorganisms still surviving or through qualitative modifications of the viable organisms themselves.

On the other hand, the experiments again bring out the value of reinoculation in establishing the viability and activity of the microorganism causing poliomyelitis.[3] The virus of this epidemic disease is peculiar in that the injection into the monkey does not afford an immunity unless obvious symptoms of an infection appear. An unsuccessful inoculation increases susceptibility to a subsequent

[3] Flexner, S., Noguchi, H., and Amoss, H. L., *J. Exp. Med.*, 1915, xxi, 91.

injection of the same relatively ineffective material. It seems as if an accumulation of the virus takes place until the quantity or number of viable microorganisms becomes sufficient to induce an infection. Repeated inoculations, therefore, rather lead to infection than to specific protection. This phenomenon was noted first with the active monkey virus used in subminimal doses for purposes of immunization[4] and later with the globoid bodies[5] of which a single inoculation failed, but repeated injections sufficed to cause experimental poliomyelitis.[3]

The demonstration of the survival of the virus in the presence of a weak antiseptic compound for the long periods given may have a bearing on the epidemiology of poliomyelitis. The reappearance in a given place of epidemic disease is usually ascribed to a new importation of virus, when possibly the explanation is sometimes to be sought in the persistence and survival of specimens of the virus which flourished previously in that or a neighboring community.

CONCLUSIONS.

The virus of poliomyelitis contained within the spinal cord and medulla of human beings and monkeys withstands glycerolation for many years. The specimens tested had been kept for 4 and 6 years respectively in 50 per cent glycerol at refrigerator temperature.

The symptoms and lesions caused by this virus are identical with those produced by that contained in the more recently collected spinal cord and medulla.

The specimens had lost a part of their activity under the conditions described, necessitating larger and repeated doses to induce infection. Whether this difference is due merely to quantitative reduction in number of viable microorganisms or to qualitative alterations under the influence of the mildly antiseptic glycerol has not been determined.

An ineffective inoculation of tissues containing the virus does not increase resistance, but rather diminishes it, so that a subsequent injection, inadequate in itself, may cause experimental poliomyelitis.

This power of survival under adverse conditions may not be without

[4] Flexner and Lewis, *J. Am. Med. Assn.*, 1910, liv, 1780.

[5] Flexner, S., and Noguchi, H., *J. Exp. Med.*, 1913, xviii, 461.

significance in respect to the recrudescence of poliomyelitis in a given locality and after a lapse of years. Hitherto this phenomenon has been accounted for by assuming a fresh importation of a virus of pronounced pathogenic power. It is possible that the explanation in some instances resides in the renewed activity of specimens of the virus surviving from a previous epidemic, while in other instances a fresh introduction actually takes place from a remote focus of infection.

The infectious nervous tissues employed in these experiments did not yield in culture streptococci or other ordinary bacteria.

EXPLANATION OF PLATES.

PLATE 40.

FIG. 1. Spinal cord of the monkey in Experiment 1, showing perivascular infiltration of meningeal and intramedullary vessels and focal accumulations of lymphoid cells. × 62.

PLATE 41.

FIG. 2. Spinal cord of the monkey in Experiment 2, showing perivascular and diffuse lymphocytic infiltration of the white and gray matter. × 62.

PLATE 42.

FIG. 3. The same as Fig. 2. Perivascular infiltrative necrosis of nerve cells and neurophagocytosis. × 400.

PLATE 43.

FIG. 4. Intervertebral ganglion of the monkey in Experiment 2, showing focal accumulations of lymphoid cells. × 62.

FIG. 1.

(Flexner and Amoss: Survival of Poliomyelitic Virus.)

FIG. 2.

(Flexner and Amoss: Survival of Poliomyelitic Virus.)

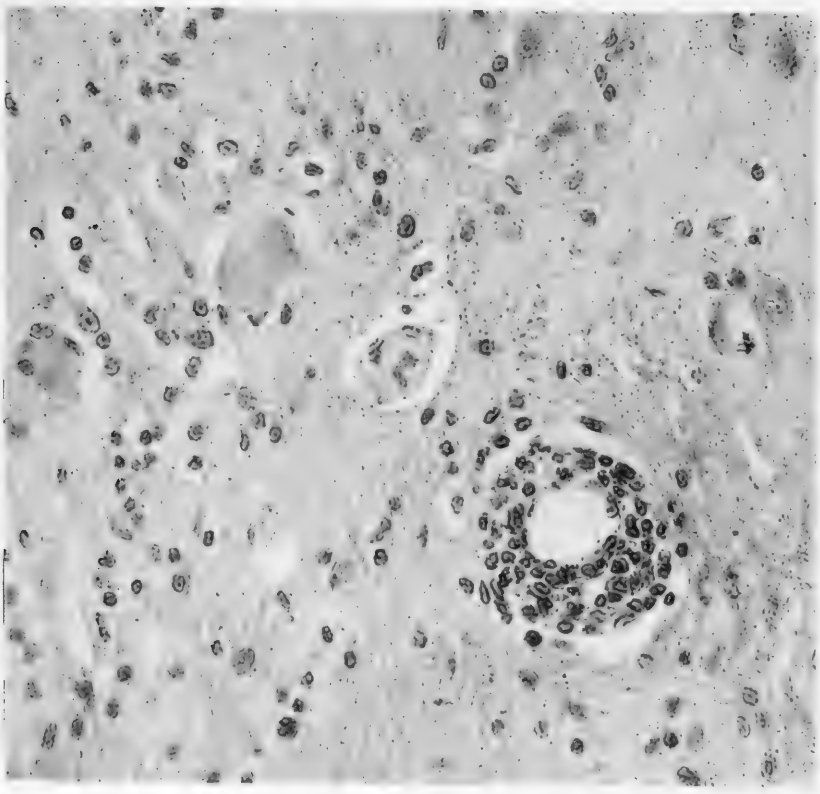

FIG. 3.

(Flexner and Amoss: Survival of Poliomyelitic Virus.)

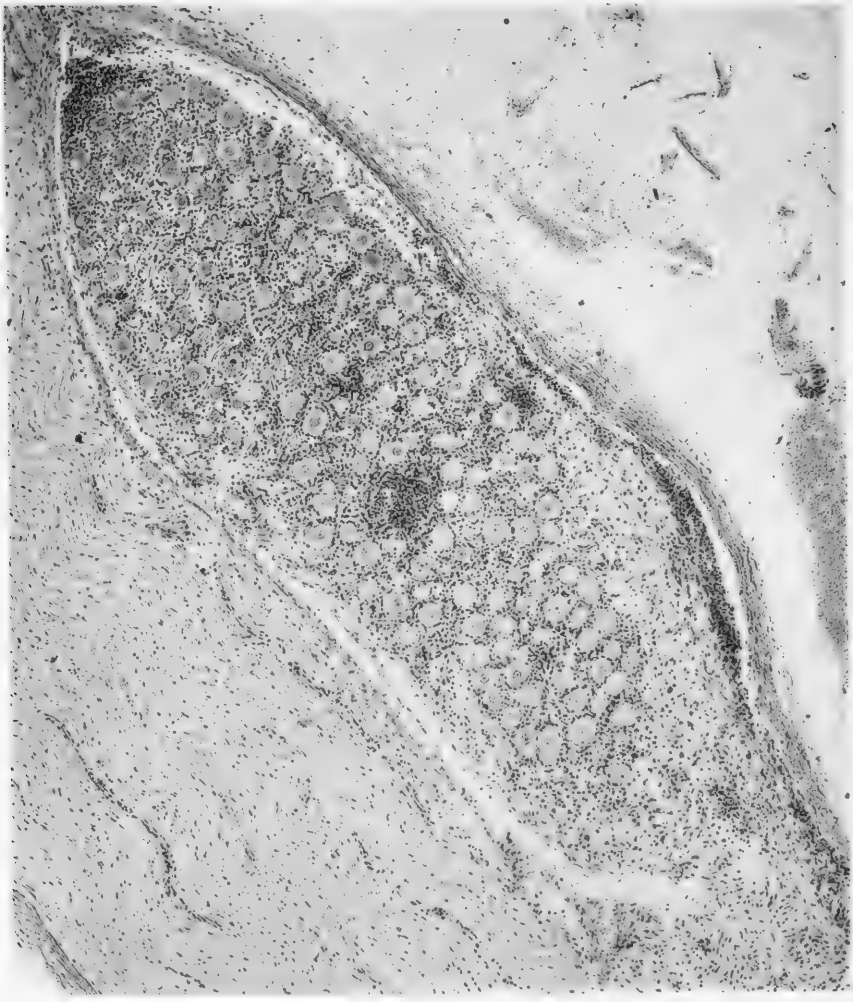

FIG. 4.

(Flexner and Amoss: Survival of Poliomyelitic Virus.)

THE CULTIVATION AND IMMUNOLOGICAL REACTIONS OF THE GLOBOID BODIES IN POLIOMYELITIS.

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PLATES 44 TO 46.

(Received for publication, December 5, 1916.)

The microoorganism described by Flexner and Noguchi[1] under the name of globoid bodies has been shown to bear a definite relation to epidemic poliomyelitis. The bodies are obtained in cultures with great difficulty and thus far only those who have employed the original method of cultivation have been rewarded with successful results. This is significant in respect to the etiology of poliomyelitis, for, while a variety of ordinary bacteria easily cultivatable has been obtained by various workers, the globoid bodies represent a peculiar species and thus far alone have sufficed to produce typical poliomyelitis in monkeys by intracerebral inoculation.

The globoid bodies may be said in general to have fulfilled Koch's law of causation. They have been found repeatedly in the lesions of poliomyelitis in man and the monkey; they have not been detected in lesions or conditions other than poliomyelitis; they have sufficed to reproduce in several instances the experimental disease in monkeys; and they have been recovered in cultures from the lesions thus produced.

But the globoid bodies have often not been obtained in cultures, even with Noguchi's technique.[2] At first sight this fact seems to cast doubt on the nature of the globoid bodies and to give rise to the question whether they are not occasional and hence not necessary accompaniments of the lesions of poliomyelitis, or, in other words, whether they constitute the microbic cause of poliomyelitis. The doubts raised by this question can at the outset be met by pointing

[1] Flexner, S., and Noguchi, H., *J. Exp. Med.*, 1913, xviii, 461.

[2] Noguchi, H., *J. Exp. Med.*, 1911, xiv, 99.

to the successful inoculations, although they are not numerous. The monkeys so inoculated developed typical poliomyelitis both symptomatically and histologically. Moreover, the nervous tissues of these animals, like the filtered virus, reproduced poliomyelitis in monkeys. Filtrates of the spinal cord and medulla and of the lesion at the local site of injection of the cultivated globoid bodies were characteristically actively infectious. Moreover, the cultures in different generations set up typical experimental poliomyelitis, and as long as 20 months after isolation and not only after a single inoculation, when most active, but subsequently, after repeated inoculation, when the activity was somewhat diminished.[3]

The globoid bodies, moreover, have been detected directly under the microscope in the nervous tissues of human beings and monkeys, the subjects of epidemic poliomyelitis,[1] and in one instance in films prepared from the circulating blood of a monkey experimentally infected with the filtered virus,[4] and in conformity with this observation they have been cultivated in one instance from the circulating blood of a monkey injected with a virus filtrate.

To one familiar with the properties of the filtered virus, this is significant. The virus filtrate does not withstand well thermostat temperatures and the presence of colloidal substances possessing absorptive power.[5] Under these conditions its activity is totally lost in from a few hours to a day or two. An injection of relatively inactive filtrate virus in monkeys does not produce resistance, but either leaves the animal unchanged immunologically or induces a state of heightened susceptibility to which, upon subsequent injection of the same virus, days or weeks later, the animal responds with typical poliomyelitic paralysis. The globoid bodies have been shown to be capable of behaving in precisely the same way.[3]

It should be recalled that the technique employed in the cultivation of the globoid bodies is based upon Noguchi's method of cultivating *Treponema pallidum*.[2] The conditions surrounding the cultivation of the two microorganisms directly from infected tissues are strikingly similar. Experience with the one is almost invaluable

[3] Flexner, S., Noguchi, H., and Amoss, H. L., *J. Exp. Med.*, 1915, xxi, 91.

[4] Amoss, H. L., *J. Exp. Med.*, 1914, xix, 212.

[5] Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1915, xxi, 509.

for success with the other. The presence of the *pallidum* in large numbers in the testicular lesions of rabbits is readily demonstrated under the dark-field microscope and by stained preparations. But bacteriologists who have attempted for the first time to cultivate the *pallidum* directly from the testicle have usually met with many difficulties and not infrequently have failed entirely.

Microorganisms as highly parasitized as the *pallidum* and the globoid bodies appear to require special conditions for growth in artificial cultures. As yet the latter have not been secured directly in a solid medium. In sharp contradistinction to this is the relative ease with which subculture succeeds, although not in every instance. Certain cultures both of the *pallidum* and the globoid bodies do not lend themselves to subculture, probably because the necessary conditions of multiplication have not been maintained in the artificial media.

Once cultivation of the strains is established, the organisms become, in time, more and more saprophytized and, as will be described in this paper, relinquish requirements, such as the presence of fresh animal tissue, indispensable at the outset. When this state arises, pathogenicity has, as a rule, been completely lost. On the other hand, as the medium has now become less complex, the conditions for the carrying out of immunological reactions have become more favorable. In one respect no change has been noted in the nature of the globoid bodies: they have remained anaerobic.

Cultivation.

When a strain of the globoid bodies has been accustomed to artificial cultivation, which usually requires several transplantations of the first cultures, growth is more readily and abundantly obtained. Apparently an adaptation to the new conditions gradually takes place. It is at first impossible to obtain large cultures, mass cultures so called; however, after several generations they may be grown in Erlenmeyer or Florence flasks of suitable size, in a medium consisting of ascitic fluid and agar containing a fragment of rabbit kidney. After congelation the substratum is overlaid with bouillon which in turn is covered with a layer of sterilized paraffin oil.[3] The growth

first takes place throughout the substratum and then extends into the bouillon, which, however, does not become turbid. The organisms appear not to multiply in the broth, but give rise to a sediment overlying the agar base. Later a mass may be obtained by collecting this sediment from several flasks. Since the bouillon will not support the growth of globoid bodies, degenerations set in quickly, the organisms showing striking irregularities in size and staining property.

The tests were carried out with four strains of the globoid bodies obtained as follows: Two cultures were original Noguchi strains obtained in 1914 from specimens of M. A. virus[1] which had been passed through many monkeys; the other two strains were obtained by the author in 1915, one from the M. A. virus, and the other from monkeys inoculated with a sample of human virus. The four cultures were indistinguishable morphologically and biologically. When they were subjected to the modification of culture media to be described, two strains had been under artificial cultivation for 2 years and the other two for 1 year or longer.

Kidney.—The indispensable animal tissue employed in the original cultivations has been fresh rabbit kidney. Noguchi believes that the tissue performs the double function of withdrawing the last traces of oxygen from the medium and of providing an essential nutritious element. Growth first appears about the tissue fragment and then extends peripherally. The tests made relate (*a*) to boiled kidney and (*b*) to dispensing with the fragment of organ altogether.

(*a*) The kidney fragments are dropped into boiling sterile distilled water and kept there until coagulation is completed. They are employed in the manner of fresh fragments in the semisolid ascitic agar medium. All the cultures multiplied, but the growth was less luxuriant than with the fresh organ.

(*b*) Growth has also taken place in complete absence of the tissue fragment, but it is even less abundant than in the presence of the coagulated tissue.

The saprophytized cultures have, therefore, become essentially altered in their extreme oxygen and food requirements. They appear to be less sensitive to oxygen and less exacting nutritionally.

Broth and Ascitic Fluid.—None of the cultures multiply in broth, even in the presence of the kidney fragment, and while some growth

is obtained in pure ascitic fluid, it is feeble. When the broth and ascitic fluid are mixed in equal proportion, growth is more abundant. The addition of 1 per cent dextrose does not improve the growth and no acid is formed. Ascitic fluid inactivated at 56°C. is unsuitable for the original cultivation of the globoid bodies, but the adapted cultures flourish equally well in the inactivated fluid.

Blood Serum.—Blood serum is not suitable for obtaining original isolations. In the presence of the kidney fragments the adapted cultures multiply freely in monkey serum, less in horse and rabbit serum, and least in human serum. The omission of the kidney has the effect of diminishing materially the amount of growth and wiping out the qualitative distinctions of the several sera. A 1:5 dilution of the sera with bouillon neither favors nor hinders this growth.

The saprophytized cultures have, therefore, undergone fundamental changes in growth requirement. The changes have produced insignificant alterations in morphology. On the whole, the saprophytized globoid bodies are somewhat less tenuous, perhaps a trifle larger, and stain more deeply with Giemsa and other stains. Their reaction to Gram's stain is also somewhat irregular, depending upon the medium employed, but they remain essentially Gram-negative. Combined artificially with cultures of streptococci and stained either with Giemsa's or Gram's stains, the distinction is striking (Fig. 1). None of the saprophytized cultures displayed pathogenic power for monkeys. Large quantities were injected intravenously into rabbits without producing any detectable ill effect. Since the cultures could now be obtained free of human protein, they were better adapted to immunological studies.

Immunology.

Our knowledge of immunological reactions in poliomyelitis is small. The one biological test for determining the presence and perhaps the amount of immune substances is that of neutralization.[6] From the fact that the blood serum of recovered human beings is protective and probably curative in poliomyelitis, it is also assumed that immune bodies are present in the fluid.

[6] Landsteiner and Levaditi, *Compt. rend. Soc. biol.*, 1910, lxviii, 311.

The particular object of this study of the immunological reactions of the globoid bodies was additional information on their relation to poliomyelitis in man and the monkey infected with the filterable virus.

This investigation was begun with considerable doubt of obtaining a clear-cut result. The difficulties surrounding the usual immunological tests with *Treponema pallidum*, with the cultural conditions of which the globoid bodies exhibited so many analogies, were before us.[7] Rabbits and monkeys were injected with the globoid bodies in order to develop antisera which might show agglutination and complement deviation, and the sera of human beings and monkeys which had recovered from attacks of poliomyelitis were tested against cultures of the globoid bodies. In order to increase their resistance, the monkeys, after recovery, had received subcutaneous injections of the emulsified active spinal cord and medulla of paralyzed animals which had succumbed.

Rabbits.—Two series of rabbits of three each received repeated intravenous inoculations of mass cultures of four strains. The first series received from 0.5 to 5 cc. of a heavy suspension of washed cultures of globoid bodies, and the second received injections from 0.5 to 5 cc. on 3 successive days, and were allowed to rest 7 days, according to the method of Flexner and Amoss for the rapid production of antidysenteric and antimeningococcic sera.[8]

With the exception of two animals that died of snuffles, the rabbits showed no reaction or symptoms, and at the end of 3 months they were well and had not lost weight. During and at the end of this time, the serum exhibited no differences in immunological reactions when compared with the normal except that in two rabbits there was slight fixation, and in one 0.1 cc. completely fixed 0.04 cc. of guinea pig complement in the presence of 0.2 cc. of antigen prepared from mass cultures. 2 cc. of the serum which exhibited the power of complement fixation, however, failed to neutralize 0.1 cc. of the filterable virus.

It is noteworthy that no clinical or immunological effect was produced in rabbits. They did not become sensitized to the globoid

[7] Zinsser, H., Hopkins, J. G., and McBurney, M., *J. Exp. Med.*, 1916, xxiv, 561.

[8] Flexner and Amoss, *J. Exp Med.*, 1915, xxi, 515. Amoss. H. L., and Wollstein, M., *J. Exp. Med.*, 1916, xxiii, 403.

bodies, nor did they respond, with one exception, with the development of detectable antibodies.

Monkeys.—Eleven monkeys received regular weekly injections of mass cultures of the fourstrains of the globoid bodies over a period of 2 years, and their serum was tested at intervals during that time. Of the eleven monkeys two received 5 cc. of a heavy suspension of the mass cultures (one recently, the other remotely isolated) intraspinally each week for 1 year, and intravenous injections of the same amounts weekly for another year. Three received 12 intraspinal injections and 28 intravenous injections; two received 5 injections intraspinally, and 35 intraperitoneally; two received a single injection intracerebrally and multiple injections intravenously; and two received intraperitoneal and intravenous injections.

Only the monkeys receiving the cultures subdurally exhibited symptoms suggestive of poliomyelitis which never progressed, however, beyond the stage of excitability, ruffling of the fur, and staccato cries.

The serum of only two monkeys, those receiving repeated intraspinal injections, developed the power of fixing complement. All the sera to a greater or less degree inhibited the growth of the globoid bodies and produced the thread reaction described below.

As has been indicated, the monkeys that received cultures of the globoid bodies over a considerable period of time were not wholly indifferent to the injections. They were refractory like the rabbits, but in two instances a reaction with antibody production was apparent. The reaction may be considered as affecting agglutination and complement deviation.

Since the globoid bodies grow in loose masses in fluid media, the ordinary methods of testing for agglutination were not satisfactory. The employment of normal and immune monkey serum in the medium of cultivation yielded sharper effects. Cultures were made in a medium composed of 1 part of the serum to be tested plus 4 parts of neutral broth.

In the presence of kidney tissue no difference can be detected between the rate and amount of growth in normal serum and immune serum. However, when kidney tissue is omitted, marked differences are apparent. Serum from monkeys immunized by intraspinal or

intravenous injection of the organism over long periods produced the most marked effect in retarding the growth of the globoid bodies. The serum from monkeys which had recovered from experimental poliomyelitis induced by the filtrate virus and which were afterwards reinforced inhibited the growth, but in less degree (Fig. 2).

Morphology.

The film preparations were made in a uniform manner. They were air-dried, fixed in methyl alcohol for 1 minute, and stained over night in 1:10 Giemsa solution and by Gram's method.

The control cultures in the ascitic-fluid-broth-kidney medium yielded cultures growing in bunches with no special arrangement of individuals. When no kidney tissue is present the chain formation is more prominent and the staining is faint. The controls in normal monkey serum with broth also give large bunches of faintly staining organisms (Fig. 3).

On the other hand, the serum of monkeys injected with the active cultures produces a marked change in the size and arrangement of the globoid bodies. The chain production is intensified and they interlace, so as to resemble the Pfaundler reaction of typhoid bacillus agglutination (Fig. 4). The length of many of the chains may be inferred from one chain in which 230 individuals were counted. The individual bodies also become larger and may become elongated. The maximum alteration is produced in the first generation in the immune serum; after several transfers in immune serum a single generation in a non-immune medium suffices to restore the original grouping and size to the cultures.

The serum from recovered and reinforced monkeys retards the growth of the globoid bodies, but less than that of the monkeys treated with the cultures. The chain formation is less also and the enlargement of the individual bodies, while apparent, is also less conspicuous (Fig. 5).

Complement Deviation.

For this test the serum (*a*) of monkeys treated with the cultures of the globoid bodies, (*b*) of recovered and reinforced monkeys, and (*c*) from paralyzed cases of poliomyelitis in man was employed.

Antigen.—For the preparation of the antigen, mass cultures were used in the manner described above, in small Florence flasks, as recommended by Zinsser.[9] The mass was washed in sterile water and suspended again in sterile water. It was alternately frozen and thawed three times, heated to 56°C. for 2 hours, transferred to a mortar, and the water evaporated at 45°C. under a current of filtered air. 1 cc. of water was added and the heavy suspension ground with glass. 20 cc. of water were added and the suspension was shaken for 30 minutes. After centrifuging at high speed, the fluid was passed through a Berkefeld filter, and made isotonic by the addition of 0.9 per cent sodium chloride solution. Extracts prepared in this manner were not anticomplementary, but rapidly lost antigenic power at 4°C.

Rabbit anti-hen hemolytic system was used when monkey serum was titrated, and rabbit anti-sheep hemolytic system when human serum was tested. Monkey serum becomes slightly anticomplementary when inactivated by heat, but the removal of complement by allowing the serum to remain at 4°C. for 4 days produces no anticomplementary action.

Serum from ten monkeys which had been injected with the cultures of globoid bodies was employed. In only two instances were reactions obtained. The two positive results were with monkeys which had received eighteen intraspinal injections of a mass culture of the globoid bodies, and later twenty-one weekly intraperitoneal injections of similar material. The serum of these animals fixed two units, or 0.4 cc., of guinea pig complement in the presence of 0.1 cc. of antigen.

The sera were subsequently tested upon the active filtrate virus and failed to show neutralizing power.

The antigen which reacted to the two sera of monkeys injected with the cultures yielded no complement fixation with the sera of monkeys which had recovered from experimental poliomyelitis or with the blood taken from twenty-six human beings, chiefly children, suffering from epidemic poliomyelitis. The latter specimens were taken for the most part in the early stages of an acute attack.

Incidentally, it may be stated that antigens were prepared from human poliomyelitic glands and from the spleen, bone marrow, lymph glands, and nasal mucosa of monkeys succumbing to experimental

[9] Zinsser, H., Hopkins, J. G., and Gilbert, R., *J. Exp. Med.*, 1915, xxi, 213.

poliomyelitis, and tested against the human blood sera. The result in every instance was negative.

Finally, the saprophytized cultures of the globoid bodies were cultivated through several generations in serum from monkeys injected with globoid bodies, from monkeys recovered from experimental poliomyelitis, and from human cases of poliomyelitis. The cultures so obtained were injected intracerebrally, under ether anesthesia, into *rhesus* monkeys but in no instance was paralysis induced.

At the outset we drew attention to the similarities in conditions of culture displayed by the globoid bodies and *Treponema pallidum;* here we desire to emphasize the analogies in immunological reaction exhibited by the culture and parasitic types of the two microorganisms respectively.[7]

CONCLUSIONS.

Two additional cultures of globoid bodies, obtained from the nervous tissues of monkeys in which experimental poliomyelitis was produced, and identical with the original cultures described by Flexner and Noguchi, are reported in this paper.

The highly parasitic cultures, like *Treponema pallidum*, are refractory to artificial cultivation.

After long cultivation outside the body, the globoid bodies acquire saprophytic properties and then grow more readily and in a considerable variety of media, provided, however, that they carry a certain quantity of protein matter not denatured.

The rabbit responds slightly with the production of antibodies to the injection of cultures of the globoid bodies.

The monkey responds only occasionally under the same conditions and apparently only when the cultures are injected into the central nervous system.

This response is small and at most leads to slight reactions of agglutination and complement deviation with the cultures.

The serum obtained from monkeys recovered from experimental poliomyelitis shows even less agglutination and no complement deviation.

The maximal agglutinative and morphological changes produced in the globoid bodies cultivated in immune monkey serum are ob-

tained in the first generation; after several subcultures in the immune serum, the reversion to normal takes place in one generation in a non-immune medium.

The serum of human beings in the acute or early subacute stages of poliomyelitis gives no complement deviation with the antigen derived from the globoid bodies,

The cultivation of the saprophytized globoid bodies through several generations in immune monkey or human serum did not confer upon them pathogenic properties for monkeys.

The serum of human beings and monkeys which have survived attacks of poliomyelitis does not fix complement in the presence of antigens prepared from organs of the monkey succumbing to the experimental disease.

The globoid bodies and *Treponema pallidum* present many analogies in cultural, immunological, and pathogenic properties.

EXPLANATION OF PLATES.

PLATE 44.

FIG. 1. Film preparation of globoid bodies (violet); *Streptococcus pyogenes* (blue). Giemsa 1: 10, 16 hours at room temperature. × 1,000.

PLATE 45.

FIG. 2. A. Culture of globoid bodies in a medium composed of 4 parts of bouillon and 1 part of normal monkey serum. No kidney tissue.

B. Culture of globoid bodies in a medium composed of 4 parts of bouillon and 1 part of serum, from a monkey which had received repeated injections of mass cultures of globoid bodies. No kidney tissue.

C. Culture of globoid bodies in a medium composed of 4 parts of bouillon and 1 part of serum, from a monkey which had recovered from experimental infection with the filterable virus. No kidney tissue.

PLATE 46.

FIG. 3. Film preparation from Tube A (Fig. 2). Giemsa stain 1: 10, 16 hours at room temperature. × 1,000.

FIG. 4. Film preparation from Tube B (Fig. 2). Giemsa stain 1: 10, 16 hours at room temperature. × 1,000.

FIG. 5. Film preparation from Tube C (Fig. 2). Giemsa stain 1: 10, 16 hours at room temperature. × 1,000.

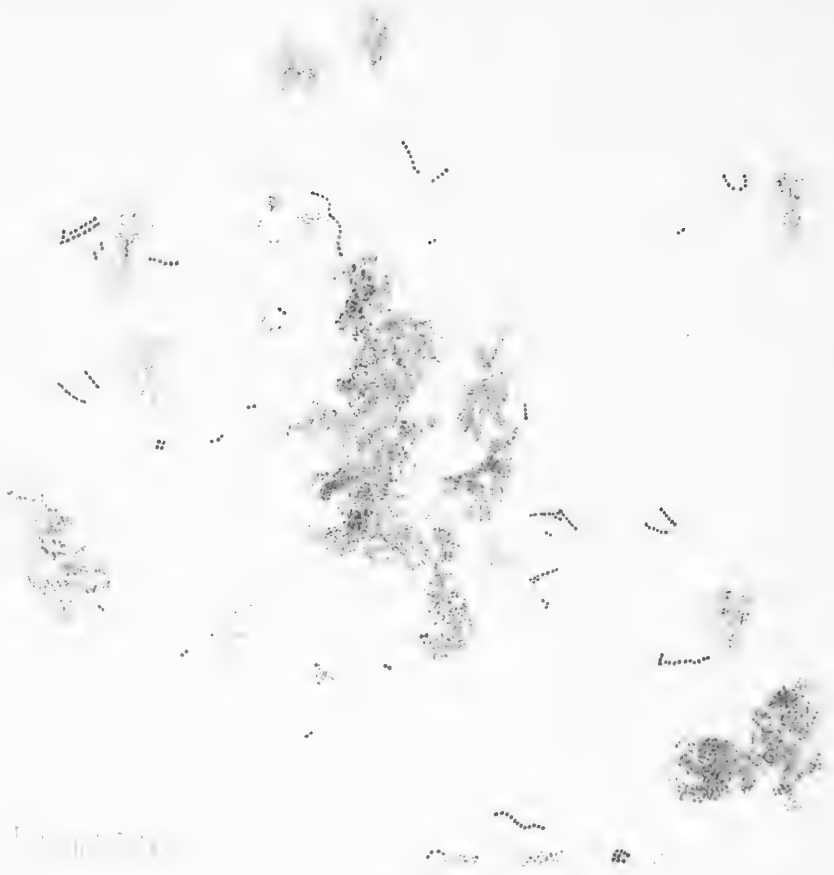

FIG. 1.

(Amoss: Globoid Bodies in Poliomyelitis.)

FIG. 2.

(Amoss: Globoid Bodies in Poliomyelitis.)

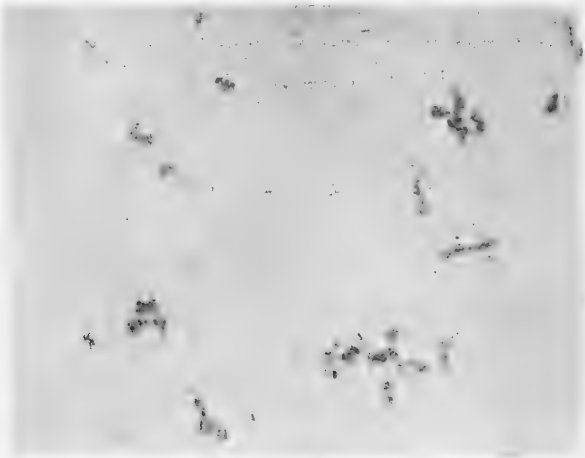

FIG. 3.

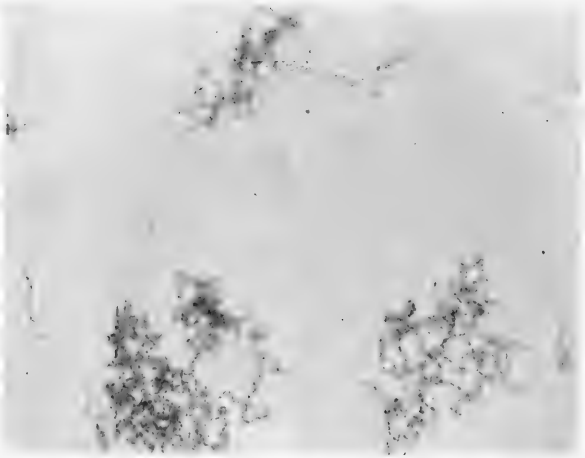

FIG. 4.

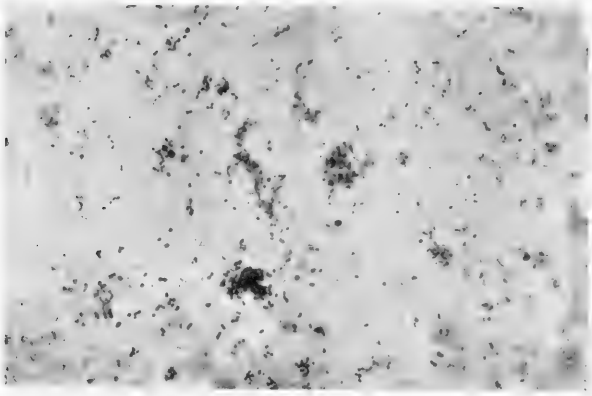

FIG. 5.

(Amoss: Globoid Bodies in Poliomyelitis.)

THE PATHOLOGIC EFFECTS OF STREPTOCOCCI FROM CASES OF POLIOMYELITIS AND OTHER SOURCES.

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PLATES 47 TO 53.

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The etiology of epidemic poliomyelitis has again become one of the debated questions in pathology. While it is not denied that the filterable virus[1] is the inciting cause of the disease in man and the monkey, the particular question which has now arisen is the relation of the streptococcus to the filterable organism.

Several authors[2] have recently reported almost simultaneously on the streptococcus as capable of setting up, on inoculation into animals, clinical and pathologic states which they identify with poliomyelitis in man and the experimental poliomyelitis induced in monkeys by inoculation of the filtered virus. A variety of animals has been given as subject to inoculation with streptococci; namely, rabbits, guinea pigs, dogs, cats, sheep, and monkeys. The streptococci reputed to produce these effects have been cultivated from the throat, tonsils, blood, and cerebrospinal fluid[3] from clinical cases of poliomyelitis, and from the tissues of the central nervous system, cerebral ventricular fluid, intervertebral ganglia, and lymph nodes of cases at autopsy. The most characteristic effect noted in the inoculated animals was paralysis of a flaccid type. This condition is stated to follow various modes of inoculation, but to result most frequently when the cultures are injected into veins.

[1] Flexner, S., and Lewis, P. A., *J. Am. Med. Assn.*, 1909, liii, 2095. Landsteiner, K., and Levaditi, C., *Compt. rend. Soc. biol.*, 1909, lxvii, 787.

[2] Mathers, G., *J. Am. Med. Assn.*, 1916, lxvii, 1019; *J. Infect. Dis.*, 1917, xx 113. Rosenow, E. C., Towne, E. B., and Wheeler, G. W., *J. Am. Med. Assn.* 1916, lxvii, 1202; *Science*, 1916, xliv, 614; *J. Am. Med. Assn.*, 1917, lxviii, 280 Nuzum, J. W., and Herzog, M., *J. Am. Med. Assn.*, 1916, lxvii, 1205. Nuzum J. W., *ibid.*, 1916, lxvii, 1437; 1917, lxviii, 24.

[3] Abramson, H. L., *J. Am. Med. Assn.*, 1917, lxviii, 546. The recent report of Abramson on approximately 1,200 fluids from acute cases is pertinent: "Except for a few evident contaminations, our cultures remained sterile."

The findings of these authors conflict in several points with those of previous investigators in this country and abroad. In the first place, the extensive investigations of Flexner and his coworkers, Levaditi and Landsteiner,[4] Leiner and von Wiesner,[5] and Kling, Pettersson, and Wernstedt[6] seemed to exclude ordinary bacteria as the inciting agents of epidemic poliomyelitis. The bacteriological studies carried out at various times and in different countries previously resulted, with one exception to be mentioned, either in obtaining no growths whatever or, as in the case of Geirsvold,[7] of obtaining from the cerebrospinal fluid an ordinary micrococcus which was believed to be a contaminant derived probably from the skin. This does not mean that occasionally bacteria were not cultivated from tissues removed post mortem from human beings and even from inoculated and paralyzed monkeys; but the stricter the conditions of bacteriological asepsis, the less frequently were the cultures obtained. The exception alluded to is the report of the cultivation of a minute, strictly anaerobic organism, globoid bodies so called, by Flexner and Noguchi[8] both from human and monkey tissues.

In the next place, the findings differ essentially with regard to the classes of animals subject to infection with the filterable virus. The only species of animals found to be regularly subject to inoculation are monkeys. Experimental poliomyelitis in the monkey is identical with the disease appearing in man. The clinical symptoms are the same, and the essential lesions in the nervous system indistinguishable. In one unmistakable instance[9] and in an instance not as certainly proven,[10] young rabbits have succumbed to inoculation of nervous tissues carrying the poliomyelitic virus. Neither the symptoms nor the lesions of the nervous system in these animals corresponded with poliomyelitis in man and the monkey.

In still another way are the older and these newer findings in conflict. The recent authors have found streptococci cultivated from a wide variety of tissues and fluids effective on inoculation. Of all the older investigators, only Krause and Meinicke[11] claim to have succeeded in producing infection with blood, cerebrospinal fluid, and various extranervous organs taken from human poliomyelitis.

[4] Levaditi and Landsteiner, *Compt. rend. Soc. biol.*, 1910, lxviii, 311.

[5] Leiner, K., and von Wiesner, R. R., in Zappert, J., von Wiesner, R. R., and Leiner, K., Studien über die Heine-Medinsche Krankheit (Poliomyelitis acuta), Leipsic and Vienna, 1911, 137.

[6] Kling, C., Pettersson, A., and Wernstedt, W., *Communications Inst. méd. État à Stockholm*, 1912, iii, 5.

[7] Geirsvold, M., *Norsk Mag. Lægevidensk.*, 1905, iii, 1280.

[8] Flexner, S., and Noguchi, H., *J. Exp. Med.*, 1913, xviii, 461.

[9] Marks, H. K., *J. Exp. Med.*, 1911, xiv, 116.

[10] Rosenau, M. J., and Havens, L. C., *J. Exp. Med.*, 1916, xxiii, 461.

[11] Krause, P., and Meinicke, E., *Deutsch. med. Woch.*, 1909, xxxv, 1825

It is noteworthy that they also employed rabbits, which they believed to be susceptible to the infection. This work has not been confirmed, except to the extent which the relatively few successful experiments in rabbits by Marks and possibly by Rosenau may be regarded as providing confirmation. But the conditions under which the rabbits succumb are so different from those observed in poliomyelitis that Marks felt obliged to determine the presence of the virus in rabbits by inoculating their nervous tissues into monkeys. The characteristic symptoms and lesions developed. He was able to transmit the virus of poliomyelitis through six series of rabbits. It was then lost.

Finally, the published reports of Mathers, Herzog, and Nuzum, and Rosenow and his coworkers contain descriptions and pictures of lesions occurring in the rabbit and monkey succumbing to the streptococcus infection which they identify with the typical lesions present in poliomyelitis in the central nervous tissues of man and the monkey. This is quite a delicate point to pass upon. Nevertheless it is essential, since the lesions of the nervous tissues in poliomyelitis are particularly characteristic. The descriptions are more convincing than the pictures, which are indeed disappointing. As a matter of fact, on the basis of the published illustrations dealing with lesions in the nervous organs, the suspicion is inevitably aroused that the pathologic processes induced by the streptococci are of another order from those occurring in human cases of poliomyelitis or arising in monkeys from the inoculation of the filtered virus.

We have therefore been led, by the intrinsic importance of the subject, to study streptococci in the manner described by these authors. But we have extended this experimental study to include with streptococci obtained from poliomyelitic patients, streptococci from a variety of other sources.

Origin and Properties of the Streptococci.

Poliomyelitic.—Cultures were made from the tonsils of twenty-seven cases of poliomyelitis, and from four pairs of tonsils extirpated during life and one pair removed at autopsy. The cultures were made both from the surface and the interior of the crypts in living cases and from the surface and the interior of the excised organs. The period of the disease at which the cultures were made varied from 3 days after onset to several weeks of convalescence.

Non-Poliomyelitic.—The cultures for comparison with the preceding were obtained (*a*) from the tonsils of children in an institution in which no case of poliomyelitis occurred during the New York epi-

demic (1916), (*b*) from tonsils extirpated at one of the large clinics of the city,[12] (*c*) from tonsils excised in private hospitals from adults and children suffering from cardiac or renal disease, and (*d*) from carious teeth removed because of root infection.

Cultural Properties.—The cultures were made on ascitic-fluid-glucose-agar and in ascitic-fluid-glucose-broth. In almost every instance the streptococcus was the predominating organism, in several instances the only organism obtained. No distinction in this respect was observed between poliomyelitic and non-poliomyelitic materials. In every instance subcultures of the second or at most of the third generation yielded pure growths.

The streptococci grew luxuriantly on the media mentioned, and on rabbit blood agar and in rabbit blood broth. The ascitic fluid cultures, after 24 hours' incubation at 37°C., usually contained a sediment at the bottom and upon the sides of the tube, the supernatant fluid being cloudy. The colonies in solid media resemble both those of pneumococcus and of streptococcus. About 1 in 20 of the cultures produced definite zones of hemolysis on the blood agar medium. The rest produced either no, or very slight hemolysis. But they caused a green discoloration of the medium indistinguishable from that produced by typical pneumococci.

The grouping of the organisms was chiefly as diplococci, but short chains were not infrequent. None could be classed as long-chain streptococci. Thirty-nine separate cultures were tested for fermentation. 54 per cent fermented inulin, 90 per cent raffinose, 89 per cent salicin, and 17 per cent mannite. Of these, 8 per cent caused definite, and 13 per cent slight hemolysis.

The morphology was affected by the conditions of cultivation. In rabbit blood broth the forms were quite regular and like streptococci. They became more irregular in the ascitic fluid media. Individuals, often in the same chain, were larger or smaller and more or less elongated. Under strictly anaerobic conditions many individuals appeared of smaller size than in the aerobic cultures.

[12] We are greatly indebted to Dr. Cornelius G. Coakley for many specimens which were removed at his clinic at Bellevue Hospital.

EXPERIMENTAL.

Practically the same varieties of animals were inoculated as those employed by other recent workers in this field. Intravenous injection was the usual manner of inoculation; but in special instances intracerebral injection, under ether anesthesia, was resorted to. The plan was to inoculate the earliest possible generations. Hence, in 10 per cent of the experiments the first cultivation generation was inoculated, in 70 per cent the second, and in 20 per cent the third generation. Usually the cultures inoculated had been grown in ascitic-fluid-glucose-broth or upon the corresponding agar; in some instances the blood broth or agar cultures were injected.

Guinea Pigs.—Twelve guinea pigs ranging in weight from 400 to 500 gm. were injected intravenously with from one-half to two cultures derived from cases of poliomyelitis. In no instance did any signs of infection appear. Hence this species of animals was not employed further.

Cats.—Four young animals ranging in weight from 500 to 700 gm. received intravenous injections of one to two cultures. One cat died on the 4th day. No paralysis or other severe symptoms had been detected. The surface of the brain yielded pure cultures of the injected streptococci. Neither brain nor spinal cord showed visible lesions, and sections prepared from both contained no pathologic changes. The remaining three animals remained well.

Dogs.—Sixteen young dogs were each given intravenously one-half to one culture per kilo of body weight from cases of poliomyelitis. Of these, one animal developed paralysis, four developed meningitis, and one an arthritis. The other dogs developed no symptoms except vomiting and diarrhea from 1 to 2 hours after inoculation. Two protocols follow.

Dog 1.—Weight 2.75 kilos.

Nov. 17, 1916. The animal received intravenously three slant cultures from an acute case of poliomyelitis in the second generation. Nov. 18. Animal weak; does not stand; respiration rapid and shallow. Nov. 19. Left hind leg flaccid; left fore leg weak. Nov. 20. Animal stronger. Paralysis continues. Nov. 21. No change. Nov. 22. Died during night.

Autopsy.—The jejunal portion of the intestine was intussuscepted and gangrenous. The meninges of the brain and spinal cord were injected and contained a gelatinous exudate. Stained films showed many streptococci being phagocyted and many free pus cells. A pure culture of streptococci was obtained from the exudate; the heart's blood and other organs gave no growth. Sections of the central nervous organs showed multiple abscesses in the cerebrum, pons, medulla, and white matter of the spinal cord. No lesions characteristic of poliomyelitis were present.

Dog 2.—Weight 2.5 kilos.

Nov. 24, 1916. The animal received three slant cultures of a streptococcus in the second generation derived from the tonsil of a poliomyelitic patient on the 3rd day of illness. Nov. 26. Drowsy; does not eat. Nov. 27. Weak; no paralysis. Died at 12 noon.

Autopsy.—Except for the central nervous system the organs were normal. The meninges contained an excess of slightly purulent fluid. Films showed pus cells and Gram-negative bacilli but no streptococci. The bacilli were being phagocyted. Only the bacillus was obtained in culture; the heart's blood and meninges yielded no streptococci. The cultural properties of the bacillus placed it in the group of *B. coli.* Sections of the brain and cord showed the existence of acute meningitis, but no lesions of the substance of either.

Rabbits.—A much larger number of rabbits was used than of any other species. The reason for this is that rabbits were chiefly used in the work recently reported. The rabbit, is, of course, known to be susceptible to experimental streptococcus and pneumococcus infection, and has been studied with reference to the former more than any other animal. Theoretically, therefore, the course and varieties of streptococcus infection in the rabbit should be well understood. In many ways they are; but this study illustrates the manner in which concentrated attention upon a particular feature of a pathologic process is capable of disclosing a number of unexpected facts. What is vastly more difficult, however, than even this detection is the proper interpretation of the new observations.

In the first place, rabbits alone, of all the varieties of animals studied by us, were inoculated with cultures from other than poliomyelitic sources. 78 animals received intravenous injections of cultures derived chiefly from the tonsils of poliomyelitic patients and 76 received intravenous injections of cultures derived from sources other than poliomyelitis. Table I summarizes the cases of paralysis and menin-

gitis and gives a record of the instances of extranervous infections.[13] A small number of individual protocols follows.

TABLE I.

Paralysis and infections.	Streptococci from poliomyelitic patients.	Streptococci from non-poliomyelitic patients.
Paralysis	4	4
Meningitis	14	15
Joint infections	13	14
Abscess in kidney	10	12
Septicemia	11	16
Abscess in skeletal muscles	5	3
Pericarditis	4	3
Endocarditis	5	4
Totals	66	71

Streptococci from Poliomyelitic Cases.

Of the 78 inoculated rabbits, 43 died and 35 still survive. Of the former number 4 developed flaccid paralysis of the limbs (Figs. 1 and 2). 18 other rabbits showed impairment of motion in one or more legs; but in these animals arthritis, tenosynovitis, or general weakness was discovered to be the cause. Daily or twice daily inspection of the animals was found advisable, because a rabbit would frequently show inability to use a leg freely without any apparent lesion, and then a day or two later it would exhibit an acute arthritis in the affected limb, often still present at the autopsy made at a later date.

Distribution of Lesions in the Rabbits Inoculated with Streptococci from Poliomyelitic Cases.[13]

Flaccid paralysis.	Meningitis.	Infection outside nervous organs.
4	14	30

Fourteen rabbits developed clinical meningitis to which they succumbed. The symptoms were, as a rule, well marked during life (Fig. 3), and were clearly determined at autopsy (Figs. 5 and 6) and from

[13] Infections in the central nervous system and other organs often occurred in the same animal.

sections of the nervous tissues (Fig. 8), while cultures from the meninges were usually positive. The clinical appearances include opisthotonos, spasticity, and hyperesthesia. In some instances, obvious clinical symptoms were wanting, but a purulent meningitis was found at autopsy. The sections showed at times focal lesions of the central nervous tissues, usually affecting the cerebrum, cerebellum, and medulla, and pons, associated with the meningitis (Figs. 9 and 10).

Thirty rabbits exhibited infections outside the central nervous organs. These consisted most often of arthritis and periarthritis, but tenosynovitis, myositis, endocarditis, abscess of the kidney, and septicemia were also frequently present (Table I).

A few illustrative protocols will be given. The first is that of a rabbit developing paralysis.

Rabbit 1.—Weight 1.5 kilos.

Oct. 13, 1916. The growth from two ascitic-glucose-agar slants from a recent case of poliomyelitis was injected intravenously. Oct. 15. Animal restless. Oct. 16. Head drawn back and to the right; hind legs paralyzed (Fig. 2). Oct. 17. Left fore leg flaccid. 10 a.m. Died.

Autopsy.—Outside the nervous system the organs appeared normal except for a small abscess in the left kidney. The meninges were hyperemic, and the cerebellum was covered with a gelatinous exudate. Cultures from the meninges gave a pure growth of streptococcus; the heart's blood gave no growth.

Sections of the brain and cord showed purulent meningitis and slight perivascular extension of the cellular exudate along the pial vessels into the substance of the cerebrum and to a less extent of the spinal cord. The gray matter of the spinal cord and medulla and the intervertebral ganglia were free of any focal lesions. A spinal nerve root in one of the sections showed a purulent infiltration (Fig. 11).

Although this animal showed paralysis of the limbs and to that extent symptoms suggestive of poliomyelitis, lesions of the nervous tissues characteristic of that condition were absent. The only histological changes detected were in the meninges and extensions along the pial investments of the blood vessels into the brain and to a less extent into the spinal cord. What other changes might have been detected had still other portions of the brain and cord been sectioned cannot, of course, be predicted; but as segments from several levels of the spinal cord, from the medulla, pons, and cerebellum, and from

different parts of the cerebrum as well as several intervertebral ganglia were studied, it may be asserted that they would in no way have resembled the typical lesions of poliomyelitis, which are not minutely focal in character.

The question arises as to the immediate causes of the paralysis of the limb. In this instance the source can only be inferred; in others it was found in focal abscesses or necroses, associated with emboli of streptococci, to be described below. Lesions similar to these do not occur in man and the monkey in poliomyelitic infection.

The culture recovered from the meninges of this animal was passed through four other rabbits in succession. The first succumbed in 56 hours to meningitis and septicemia. Sections of the nervous organs showed purulent meningitis and focal abscesses of the brain. The second rabbit, inoculated from the previous one, developed arthritis of both wrist joints. Cultures from this animal produced meningitis in a third, and cultures from the third, septicemia in a fourth rabbit.

Three other rabbits in this series developed, as has been stated, some degree of flaccid paralysis of the limbs. The histological studies of the central nervous organs confirmed essentially the findings given in the protocol above. In each instance there was evidence of acute meningitis with extension of the inflammation along the pial membrane into the substance of the nervous organs and chiefly the cerebrum. The perivascular cellular infiltration always coexisted with and was part of the meningitis; and this in turn was sometimes attended by focal abscesses (Figs. 9 and 10). In none were lesions of the nerve tissues *per se* detected, neither diffuse interstitial infiltration nor degeneration with consequent phagocytosis of the nerve cells. The spinal cord and intervertebral ganglia were notably free of lesions. In rare instances small hemorrhages existed in the spinal cord.

Rabbit 2.—Weight 1.55 kilos.

Oct. 10, 1916. The sediment of two tubes of ascitic-glucose-broth containing streptococci in the first generation was injected intravenously. The culture was obtained from the tonsils of a poliomyelitic patient during the first days of illness. Stiffness of the legs was present the following day; that is, in less than 24 hours after inoculation. The chief clinical symptom was spasticity of the hind legs. No further symptoms developed, but death occurred 50 hours after injection.

Autopsy.—The thoracic and abdominal organs showed nothing abnormal except that the right kidney contained a small infarcted area. The meninges were hyperemic, but no changes were detected in the dissected brain and cord. No streptococci were cultivated either from the heart's blood or the central nervous organs.

The histological lesions present in the cerebrum consisted of areas of marked perivascular infiltration (Fig. 14) associated with foci of necrosis (Fig. 15). The meninges were the seat of marked infiltration—an acute meningitis. And the relation between the meningitis, perivascular infiltration, and focal necroses was readily established. But the medulla, spinal cord, and intervertebral ganglia were all free of lesions, although the meninges about them showed some degree of cellular infiltration.

In all, five other rabbits of this series showed histological lesions similar to those described. In two of these a purulent meningitis coexisted. In none were any changes present in the gray matter of the spinal cord or in the intervertebral ganglia.

Rabbit 3.—Weight 1 kilo.

Nov. 18, 1916. The growth from two ascitic agar slants of a streptococcus isolated from a carious tooth of a convalescent poliomyelitic patient was injected intravenously. Nov. 19. No symptoms had developed. Nov. 20. Pronounced opisthotonos (Fig. 3). Nov. 21. No change. Nov. 22. Meningeal symptoms still present. Rabbit weak. Nov. 23. Condition unchanged. Nov. 24, 8 a.m. Rabbit found dead.

Autopsy.—The meninges of the brain and upper portion of the spinal cord were injected. Impression films from the brain and medulla showed many streptococci and pus cells. No lesions were found in the abdominal and thoracic organs. Pure cultures of streptococci were obtained from the surfaces of the brain and medulla.

Sections of the brain and cord showed a purulent meningitis.

The protocols of the rabbits illustrate instances in which (1) flaccid paralysis of the limbs developed, (2) spasticity appeared, and (3) meningitis was the prominent symptom. The question arises whether the conditions described correspond to or resemble those present in poliomyelitis as it is known in man and the monkey.

The decision must rest not only upon the gross symptoms but even more upon the histological appearances and in part also upon still another consideration; namely, the possibility of conveying, by inoculation of the rabbit culture material, poliomyelitis to monkeys. This

last aspect of the case will be considered below in connection with the experiments made on monkeys.

In order that the symptoms and lesions in rabbits may be interpreted, it is desirable to recapitulate briefly the main symptoms and lesions of poliomyelitis in man and the monkey. Aside from indefinite signs such as fever, malaise, etc., they consist of meningitis and flaccid paralysis. In man the meningitic symptoms often precede the paralytic, but death appears never to be caused by the meningitis but always by the paralysis, affecting mainly the function of respiration. In the monkey symptoms of meningitis appear, but they are trifling and overshadowed by the paralysis. Death here, too, invariably is caused by the paralysis affecting respiration.

The lesions of the nervous organs of man and the monkey are essentially identical: first, a certain degree of meningeal infiltration, not diffuse, but confined mainly to the region of the large blood vessels, particularly about the medulla and spinal cord and slightly over the cerebrum; second, a marked degree of intramedullary perivascular infiltration affecting slightly the cerebrum, and practically constantly the medulla, spinal cord, and intervertebral ganglia. This perivascular infiltration is associated with more or less diffuse cellular invasion of the gray matter, chiefly of the medulla and spinal cord and rarely of the cerebrum. Focal cellular accumulations also occur in the ganglia, sometimes between, sometimes involving the nerve cells. Hemorrhages are larger and more frequent in man than in the monkey. The nerve cells of the spinal cord, less often of the medulla, and least often of the cerebrum, suffer degeneration and necrosis and subsequent phagocytic destruction. Similar occurrences are observed in the intervertebral ganglia. The nerve cells chiefly affected in the spinal cord are the motor, but the cells in the posterior gray matter also suffer. The lesions in the nervous tissues are not sharply focalized; the cellular invasion of the meninges is chiefly lymphocytic and never chiefly polymorphonuclear or purulent.

The lesions met with in the rabbits are sharply contrasted with these. They consist of meningitis of polymorphonuclear and usually purulent kind, either focal or general; of focal abscesses in the gray matter, most frequent in the cerebrum (Figs. 8, 9, and 10), next in the cerebellum, and very rarely in the medulla and spinal cord; of focal

areas of necrosis of nerve cells, almost exclusively in the cerebrum, surrounded by infiltration of cells of lymphocytic type (Fig. 15) and adjacent to blood vessels showing perivascular infiltration (Fig. 14), extending usually from the meninges. This last and most striking lesion, which may be called focal meningo-encephalitis, is unlike any condition observed in poliomyelitis in man and the monkey. In some instances the foci were general throughout the brain; they were very infrequent in the spinal cord. The distribution of necrotic foci and perivascular infiltration was identical. According to the plane of the section, varying pictures are obtained. Sections through the center (Fig. 15) show a necrotic mass of gray matter surrounded by a collar of mononuclear cells; sections through the periphery may show only a collection of the latter cells (Fig. 13).

Finally, preparations stained for bacteria disclose a wide difference between the findings in man and the monkey and in the rabbits under consideration. While no bacteria have been detected in the former, by the use of ordinary methods, the reverse is true of the latter. The diffuse meningitis in rabbits shows, as a rule, in impression preparations many streptococci and polynuclear leukocytes (Fig. 5). In instances of quickly developing meningitis and rapid death, the cocci are more, and the leukocytes less numerous (Fig. 6). Phagocytosis of the streptococci is common. The focal areas of meningitis also contain streptococci, although not so readily demonstrable. The focal abscesses contain streptococci in masses in the central part and scattered in the periphery (Fig. 7). Probably the central mass was derived from an embolus. In other words, the lesions present in the rabbits are such as would be produced if streptococci or other suitable microorganisms entered the central nervous tissues in such a way and in sufficient numbers to survive and set up infection and inflammation.

This last consideration led to an examination of several stock rabbits of a lot in which snuffles was prevailing. Among three of this group which succumbed to a bacillary septicemia and of which sections of the nervous organs were prepared, one was found showing an acute meningitis attended by perivascular infiltration within the cerebrum (Figs. 12 and 13) indistinguishable from the corresponding conditions present in the animals inoculated with streptococci. In

view of the common prevalence of spontaneous infections among rabbits, one is led to inquire whether the perivascular lesions may not have preexisted in the animals prior to the injection of the streptococci. This supposition has indeed just received strong support from a publication by Reasoner[14] in which he figures this precise lesion as caused by *Spirochæta pallida* localized in the cerebrum of the rabbit. We believe more than ever, therefore, that lesions of this kind, obviously not specific, may be the result of bacillary infection to which rabbits under laboratory conditions are subject. In any case, they cannot be regarded as in any degree peculiar to, and indicative of poliomyelitis, a conclusion supported also by the experiments with streptococci of other than poliomyelitic origin.

Streptococci from Non-Poliomyelitic Cases.

76 rabbits were inoculated with streptococci of non-poliomyelitic origin. Of these, 58 rabbits died in the course of the experiments. No differences could be detected between the effects of these streptococci and those obtained from cases of poliomyelitis. Even the numerical results are equivalent.

Distribution of Lesions in the Rabbits Inoculated with Streptococci from Non-Poliomyelitic Cases[13]

Flaccid paralysis.	Meningitis.	Infections outside nervous organs.
4	15	37

A few illustrative protocols follow.

Rabbit 4. Spastic, Succeeded by Flaccid Paralysis.—Weight 2 kilos.

Oct. 16, 1916. Two slant cultures were injected intravenously. The streptococcus employed was in the second generation and came from the extirpated tonsils of an adult suffering from chronic nephritis. Oct. 20. Sluggish. Oct. 21. Hind legs spastic. Oct. 22. Hind legs flaccid; animal prostrate; unable to rise. 6 hours later marked opisthotonos appeared, and 1½ hours afterwards death occurred.

Autopsy.—Except for the central nervous system, the internal organs appeared normal. The muscles about the hip joints contained abscesses from which streptococci were recovered in films and cultures. The meninges of the medulla

[14] Reasoner, M. A., *J. Am. Med. Assn.*, 1916, lxvii, 1799.

and cerebellum contained a gelatinous exudate from which, as well as from the fourth ventricle, streptococci were found in films and cultivated.

Sections from all the nervous organs showed (1) in the cerebrum and cerebellum multiple focal abscesses containing large numbers of streptococci; (2) focal purulent meningitis, in which streptococci were difficult to demonstrate; (3) the absence of lesions in the medulla, spinal cord, and intervertebral ganglia.

Rabbit 5. Flaccid Paralysis and Convulsions.—Weight 1.8 kilos.

Nov. 2, 1916. Three slant cultures were injected intravenously. The streptococcus employed was in the second generation and had been obtained from the tonsil of a child in an isolated infant home in which no case of poliomyelitis had developed. Nov. 4. Left fore leg flaccid (Fig. 4); no other symptoms. Nov. 9. No change until today. About 1 p.m. convulsions developed which became more and more violent. 2.20 p.m. Died.

Autopsy.—The abdominal and thoracic organs appeared normal. The meninges of the brain and cord were moderately congested, and an exudate was present in the lumbar region. Dissection revealed nothing. The joints and muscles of the paralyzed leg showed no abnormality. Cultures and films from the blood, nervous organs, and joints showed no streptococci.

Sections prepared from various portions of the nervous organs showed merely punctiform hemorrhages in the cervical cord, medulla, and pons.

Rabbit 6. No Characteristic Symptoms. Perivascular Lesions in the Meninges and Cerebrum.—Weight 1.6 kilos.

Nov. 6, 1916. Two slant cultures in the second generation were injected intravenously. The streptococci were obtained from the extirpated tonsils of an otherwise healthy child. Nov. 7. No symptoms. Nov. 8. Died.

Autopsy.—The surface of the kidneys contained punctiform hemorrhages; spleen enlarged; acute pericarditis. The meninges were highly congested and an exudate covered the cerebellum and medulla. Streptococci and polymorphonuclear leukocytes were present in the exudate in the pericardium and meninges. Cultures were positive.

Sections from the nervous organs showed infiltration of the meninges and about the blood vessels (Fig. 16) in the cerebrum, attended by foci of necrosis (Fig. 17).

A second rabbit inoculated with this strain developed fatal septicemia.

After what has already been said in the discussion of the results of the injection of rabbits with streptococci derived from cases of poliomyelitis, little need be added here. The essential facts seem to be (1) that streptococci derived from conditions other than poliomyelitis are capable of producing lesions and symptoms in rabbits indistinguishable from those produced by streptococci of poliomyelitic

origin; and (2) that with a sufficiently large series of rabbits, the numerical proportion of lesions tends to be identical in both instances. The general conclusion indicated by the two classes of experiments is that when large numbers of streptococci possessing a certain grade of virulence are injected into the veins of rabbits, they tend by preference to localize in the meninges, from which the cerebrum may subsequently be invaded; next to the meninges, they seek the joints, and then the voluntary muscles and other situations. In these several localizations, they set up inflammations and suppurations in which they survive for a time, but they may also die. The conditions are analogous to experimental pneumococcus infection in the dog. This animal, relatively resistant to pneumococci, will respond to very large doses by the development of a purulent meningitis and associated encephalitis.[15] In the rabbit, too virulent cultures quickly kill through septicemia unassociated with particular localizations, while avirulent cultures are quickly destroyed, and hence do not localize at all.

In other words, it is not evident that the nervous lesions of streptococcus infection in the rabbit can be identified with the specific disease poliomyelitis as we know its manifestations in man and the monkey.

Experiments on Monkeys.

In this paper the monkey has frequently been referred to as the one experimental animal in which thus far the typical symptoms and pathologic lesions of poliomyelitis in man have been reproduced. Hitherto, infection of the monkey has been accomplished by means of the filterable virus contained within the nervous and some other organs of human beings suffering from poliomyelitis, and in a few instances besides through inoculation of the anaerobic cultivated globoid bodies of Flexner and Noguchi.[8] The recent workers alluded to[2] claim to have produced typical poliomyelitis in the monkey with streptococci. Since this species supplies the decisive test, we carried out crucial experiments with it.

The method of inoculation recommended by these authors departs considerably from the one usually employed with the filterable virus.

[15] Bull, C. G., *J. Exp. Med.*, 1916, xxiv, 7.

With the latter, infection by way of the blood is difficult or impossible to achieve;[16] with the streptococci, intravenous inoculation has been preferred.

Monkey 1.—Nov. 18, 1916. A *Macacus rhesus* was given intravenously a suspended sediment from 60 cc. of ascitic-fluid-glucose-broth culture in the second generation of streptococcus obtained from the tonsils from a poliomyelitic patient. Nov. 19. Animal moves about more slowly; generally weak; no paralysis. Nov. 20, 8 a.m. Voice high pitched. 10 a.m. Legs spastic. 11 a.m. Retraction of head; marked hyperesthesia. 1 p.m. Died.

Autopsy.—The organs outside the nervous system showed nothing of importance. The meninges were congested and edematous. The substance of the brain and cord appeared normal. Films from the meninges of the brain and cord showed streptococci, pus cells, and mononuclear cells; pure cultures of streptococcus were obtained.

Sections of the brain and cord showed only a purulent meningitis. No lesions were detected in the substance of the spinal cord, medulla, pons, cerebrum, or intervertebral ganglia.

Monkey 2.—Dec. 11, 1916. A *Macacus rhesus* was given intravenously the suspended sediment from 60 cc. of ascitic-glucose-broth culture. The culture of streptococcus used in this experiment came from the tonsil of a poliomyelitic patient and had been passed through two rabbits, of which one had developed paralysis of the legs, and both at autopsy had shown purulent meningitis and focal cerebral abscesses. Dec. 12. No general effect; double panophthalmitis appearing. Dec. 13. No change in the general condition; anterior chamber and vitreous opaque and purulent. The sediment from 30 cc. of a similar culture was injected intravenously. Within 2 hours the animal became ill and weak; no paralysis was detected. Dec. 14. Died.

Autopsy.—The lungs were edematous and hemorrhagic; spleen swollen, dark, and friable; mesenteric nodes hemorrhagic. The meninges were congested; there was no visible lesion of the spinal cord or brain aside from punctate hemorrhages in the latter. Films and cultures prepared from the interior of the eyes and the fourth ventricle gave streptococci. Cultures from the heart's blood and surface of the brain were negative.

Sections of the brain, medulla, spinal cord, and intervertebral ganglia showed no lesions aside from small extravasations of blood in the cerebrum.

Since this is the type of experiment which is said by Rosenow and his coworkers to have given the most constant result, it was repeated on four other *Macacus rhesus* monkeys. In each instance some degree of depression followed the massive injections; in one instance a

[16] Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1914, xx, 249.

purulent panophthalmitis again developed. Paralysis never appeared, and all four animals recovered.

In other words, our experiments were uniform failures as far as producing in monkeys paralysis or any condition resembling poliomyelitis was concerned, by means of cultures of streptococci derived from cases of poliomyelitis and injected either directly into monkeys or first into rabbits and then into monkeys. As will be shown below, the monkeys which received massive doses of the streptococci showed no undue resistance to inoculation with the filtered virus of poliomyelitis.

Streptococci from the Poliomyelitic Spinal Cord.

The attention which has been directed to streptococci in poliomyelitis has led to the discovery that streptococci can be cultivated from specimens of the brain and spinal cord apparently of both persons and monkeys succumbing to the disease. By employing considerable portions of the spinal cord of monkeys, we have in a few instances obtained growths of streptococci from them. Only a part of the cultures made yield the cocci; many tubes remain sterile. The following protocol illustrates this point.

When moribund, a poliomyelitic monkey was etherized and portions of the brain and spinal cord and two lymph nodes were removed aseptically. The brain and cord were inoculated (*a*) in fragments and (*b*) in emulsions into ascitic-glucose-broth and agar respectively. The lymph nodes were inoculated into the fluid medium. The agar medium was inoculated while fluid and then allowed to congeal. Twenty tubes in all were inoculated. One-half the tubes were covered with paraffin oil. The incubation was at 37°C. After 48 hours two broth tubes containing emulsified brain tissue contained growths of streptococci. After 72 hours four more tubes of broth showed streptococci, of which two had been inoculated with brain emulsion, one with a brain fragment, and one with a lymph node. 14 tubes remained sterile. It is worth noting that the comminuted brain tissue alone, except for one brain fragment, yielded streptococci; the spinal cord cultures yielded none. The distribution of the streptococci in the central nervous system must at best have been quite irregular. Moreover, none of the solid cultures yielded detectable growths. A single surviving streptococcus in a fluid culture would multiply abundantly; in a solid culture it would probably be lost. The streptococci from these sources were injected intravenously into six medium sized rabbits. One rabbit died in 48 hours. The

autopsy showed a purulent meningitis. Another developed arthritis 72 hours after inoculation. The remaining four showed no symptoms.

The original streptococcus from this source was transferred to 60 cc. of ascitic-glucose-broth of which, after incubation at 37°C., the sediment was injected intravenously into a *Macacus rhesus*. No symptoms developed, and hence 48 hours later, the sediment from 30 cc. of a broth culture was also injected. Within 24 hours the animal was somewhat slow and irritable; the condition quickly passed away. No paralysis or other severe symptoms developed.

At the same time that the ascitic broth culture was prepared, another was made from the original growth in blood agar. The entire growth was washed off and injected intracerebrally, under ether anesthesia, into a *Macacus rhesus*. No symptoms of any kind developed.

The streptococcus derived from the brain tissue produced a green color in blood and otherwise resembled closely *Streptococcus viridans*, so called.

Before considering the significance of this experiment, the following additional note should be made. Several stock specimens of recently glycerolated spinal cord from monkeys which had succumbed to poliomyelitis were incubated in ascitic-glucose-broth and a specimen was obtained yielding streptococci corresponding to *Streptococcus viridans*. Recent cultures from this source were inoculated (*a*) intravenously into two rabbits, (*b*) intracerebrally, under ether anesthesia, into a *Macacus rhesus*, and (*c*) the latter test was controlled by inoculating a filtrate of the spinal cord itself into another *Macacus rhesus*. The results are significant. The rabbits each developed meningitis and kidney abscesses, without paralysis. Sections of the spinal cord and brain showed only lesions of meningitis. The monkey inoculated with the streptococcus remained well; the other inoculated with the filtrate developed paralysis and died.

The conditions presented by the foregoing experiments on monkeys should theoretically, assuming that streptococci have an etiologic relation to poliomyelitis, have been favorable to the production of paralysis and the characteristic nervous lesions in at least part of the monkeys inoculated. That in no single instance any definite infection resulted except purulent ophthalmitis, and that the streptococci derived immediately from the nervous tissues of poliomyelitic monkeys should have been inert is difficult to harmonize with the view that the streptococcus is capable of producing poliomyelitis in monkeys, especially as the filtrate from the nervous tissue of the paralyzed monkey was readily effective under circumstances in which the streptococcus obtained from the same source was entirely inactive.

Does Streptococcus Infection Protect against Infection with the Filtered Virus?

Recovery from poliomyelitis induced by the filtered virus leaves a state of resistance in monkeys indicated by insusceptibility to reinoculation[17] and by the power of the blood serum to neutralize the filtered virus.[18] Tests were made to determine whether monkeys having received several injections of cultures of streptococci from poliomyelitic sources exhibited protection by either of the foregoing reactions.

Neutralization in Vitro.—For this test two monkeys which had resisted streptococcus inoculation were taken and their immunity to the organism was increased by the following injections: to each was given intravenously the sediment from 100 cc. of broth culture of the original strain employed and then on 2 successive days sediment from 10 cc. of a broth culture of the streptococcus cultivated from the glycerolated spinal cord of a poliomyelitic monkey. 10 days after the last injection the sera of the monkeys agglutinated the streptococci in dilutions up to 1: 1,500. Normal monkey serum agglutinated the same cultures up to 1: 300.

The tests for neutralization were made by mixing 0.1 cc. of a Berkefeld filtrate prepared from a 5 per cent emulsion of active poliomyelitic virus with 1 cc. of monkey serum, incubating for 2 hours at 37°C., and keeping for 16 hours at 4°C. The injections were made intracerebrally, under ether anesthesia, into two *Macacus rhesus* monkeys.

Monkey 3.—Jan. 3, 1917. A *Macacus rhesus* was inoculated. No symptoms appeared until Jan. 10 when the right arm became flaccid. Jan. 11. The left arm was flaccid. Jan. 12. The muscles of the trunk were paralyzed. Jan. 13. The animal was etherized.

Autopsy.—The gross poliomyelitic lesions of the medulla were typical, and the sections of the spinal cord, medulla, and intervertebral ganglia were characteristic of the disease.

Monkey 4.—Jan. 3, 1917. A *Macacus rhesus* was inoculated. No symptoms developed until Jan. 7 when the animal protected the right arm. Jan. 11. Ataxia

[17] Flexner and Lewis, *J. Am. Med. Assn.*, 1910, liv, 45.

[18] Landsteiner and Levaditi, *Compt. rend. Soc. biol.*, 1910, lxviii, 311.

and pronounced tremor of head. Jan. 12. Legs flaccid; animal prostrate. Jan. 13. Etherized.

The spinal cord and medulla showed typical gross lesions and the sections were characteristic of poliomyelitis.

Protection Tests.—Three monkeys which had received large injections of streptococci from cases of poliomyelitis were tested for protection against the filtered virus.

Monkey 5.—Nov. 22, 1916. A *Macacus rhesus* received intravenously 50 cc. of ascitic-glucose-broth culture of poliomyelitic streptococci which had been passed through one rabbit. A period of depression followed, but on Nov. 24 the animal appeared normal. The streptococci from 40 cc. of a similar broth culture were injected. No marked symptoms developed. Dec. 12. 1 cc. of the filterable virus from fresh brain and spinal cord of a paralyzed monkey was injected intracerebrally under ether anesthesia. Dec. 17. Ataxia. Dec. 18. Prostrate. Dec. 19. Died.

The autopsy showed marked poliomyelitic lesions.

Monkey 6.—Nov. 26, 1916. A *Macacus rhesus* received intravenously 45 cc. of a broth culture of streptococcus of poliomyelitic origin. Nov. 28. No symptoms had appeared. The sediment from 50 cc. of culture was injected. Dec. 1. Animal weak, moves about slowly. Dec. 3. Recovered. Dec. 12. Very active. 1 cc. of a suspension of brain and spinal cord of poliomyelitic monkeys was injected intracerebrally under ether anesthesia. Dec. 15. Ataxia; tremor; double ptosis. Dec. 17. Prostrate. Dec. 21. Very weak. Etherized.

The autopsy showed typical lesions of poliomyelitis.

Monkey 7.—Dec. 6, 1916. A *Macacus rhesus* received intravenously the sediment from 60 cc. of an ascitic-glucose-broth culture of poliomyelitic streptococci, Dec. 7. Animal somewhat ill; eyes cloudy. Dec. 8. The sediment from 30 cc. of a culture was injected intravenously. Dec. 9. Moves slowly; not well. Dec. 12. Well. Jan. 23, 1917. The sediment from 100 cc. of a broth culture was injected. No effect. Jan. 24. 10 cc. of a broth culture of streptococcus obtained from the nervous tissues of a poliomyelitic monkey were injected. Jan. 25. 10 cc. of the same culture were injected as on the day before. No effect produced. Feb. 3. 0.5 cc. of a suspension of poliomyelitic spinal cord of a monkey was injected intracerebrally under ether anesthesia. Feb. 7. Both arms paralyzed. Feb. 8. Prostrate. Etherized.

The spinal cord showed marked lesions of poliomyelitis.

DISCUSSION.

The results obtained by us from the inoculation of several species of laboratory animals with cultures of streptococci from poliomyelitic patients and other sources fail to establish an etiologic relationship

between streptococci and epidemic poliomyelitis. In this respect our experiments are in sharp contrast to those of Mathers, Herzog, and Nuzum, and Rosenow and his coworkers. Not only were we unsuccessful in inciting poliomyelitis with cultures of streptococci, but we found that these organisms produce chiefly in rabbits various conditions and lesions that are absent from man and the monkey, the subjects of poliomyelitis, and the conditions and lesions result equally well from streptococci originating in persons not suffering from or exposed to the disease. In other words, they are referable not to poliomyelitis, but to streptococcus infection. That they may also be present accidentally in stock rabbits has also been shown. Finally, we have failed to detect any immunological relationship between streptococci and the filterable virus of poliomyelitis.

It may be pointed out that the mere appearance of paralysis in animals is no sufficient criterion for concluding that poliomyelitis exists or has been produced. The toxin of the Shiga bacillus, for example, frequently causes flaccid paralysis in rabbits. It may be considered as established that streptococci do sometimes cause paralysis in rabbits and possibly other animals, but our experiments show that this is determined not by the source but by the degree of virulence of the cultures. The final test indeed is the monkey, not the rabbit or other domestic animals, and we not only failed with streptococci to induce paralysis in monkeys, but we failed to render them more resistant or their blood serum neutralizing to the filtered virus of poliomyelitis.

Our failures cannot be ascribed to minor variations in technique. About 10 per cent of the streptococcus cultures from poliomyelitic cases were obtained pure in the first generation and at once inoculated; and no distinction was discovered between the lesions thus produced and those set up by the cultures in the second and third generations. There is one advantage which the earlier generations possess over the later ones; they are somewhat more virulent. Yet even the first generations possessed little infective power and enormous doses were required to produce any pathologic effect. The rapid loss of power of streptococci to produce paralysis even when passed from animal to animal is in sharp contrast with the increase occurring under similar conditions in the filtered virus. No two sets

of pathologic actions and effects could be more diverse than those observed by us to be caused by streptococci and by the filtered virus of poliomyelitis.

SUMMARY.

Streptococci cultivated from the tonsils of thirty-two cases of poliomyelitis were used to inoculate various laboratory animals.

In no case was a condition induced resembling poliomyelitis clinically or pathologically in guinea pigs, dogs, cats, rabbits, or monkeys.

On the other hand, a considerable percentage of the rabbits and a smaller percentage of some of the other animals developed lesions due to streptococci. These lesions consisted of meningitis, meningo-encephalitis, abscess of the brain, arthritis, tenosynovitis, myositis, abscess of the kidney, endocarditis, pericarditis, and neuritis.[19]

No distinction in the character or frequency of the lesions could be determined between the streptococci derived from poliomyelitic patients and from other sources.

Streptococci isolated from the poliomyelitic brain and spinal cord of monkeys which succumbed to inoculation with the filtered virus failed to induce in monkeys any paralysis or the characteristic histological changes of poliomyelitis. These streptococci are regarded as secondary bacterial invaders of the nervous organs.

Monkeys which have recovered from infection with streptococci derived from cases of poliomyelitis are not protected from infection with the filtered virus, and their blood does not neutralize the filtered virus *in vitro*.

We have failed to detect any etiologic or pathologic relationship between streptococci and epidemic poliomyelitis in man or true experimental poliomyelitis in the monkey.

[19] Many thousand cases of epidemic poliomyelitis occurred in New York and elsewhere during the past summer (1916) and many cases were subjected to autopsy. But no instance of metastatic infection and inflammation such as occur usually in streptococcus infection has been reported.

EXPLANATION OF PLATES.

PLATE 47.

FIG. 1. Rabbit. Flaccid paralysis of fore leg appearing 48 hours after an intravenous inoculation of streptococci from a poliomyelitic tonsil.

FIG. 2. Rabbit. Flaccid paralysis of both hind legs and meningitis appearing 72 hours after an intravenous inoculation of poliomyelitic streptococci.

PLATE 48.

FIG. 3. Rabbit. Meningitis and opisthotonos appearing 48 hours after an intravenous inoculation of poliomyelitic streptococci.

FIG. 4. Rabbit. Flaccid paralysis of the left fore leg appearing 72 hours after an intravenous inoculation of non-poliomyelitic streptococci.

PLATE 49.

FIG. 5. Contact film from the cerebrum of the rabbit shown in Fig. 2. × 1,000.

FIG. 6. Contact film from the cerebrum of a rabbit which survived 72 hours after an intravenous inoculation of non-poliomyelitic streptococci. × 1,000.

FIG. 7. Streptococci in brain abscesses from a rabbit which survived 72 hours after an intravenous inoculation of poliomyelitic streptococci. Gram-Weigert stain. × 1,000.

PLATE 50.

FIG. 8. Purulent meningitis. Section from the rabbit shown in Fig. 2. × 250.

FIG. 9. Cerebral abscess. Section from a rabbit which survived 72 hours after an intravenous inoculation of non-poliomyelitic streptococci from the tonsil of an adult. × 250.

FIG. 10. Cerebellar abscesses from the same rabbit as Fig. 9. × 40.

PLATE 51.

FIG. 11. Purulent neuritis. Section from a spinal nerve root of a rabbit which survived 72 hours after an intravenous inoculation of poliomyelitic streptococci. × 250.

FIG. 12. Perivascular infiltration. Cerebrum of a stock rabbit which succumbed to bacillary infection. × 250.

FIG. 13. Round cell infiltration from the same rabbit as Fig. 12. × 250.

PLATE 52.

FIG. 14. Perivascular infiltration. Cerebral cortex of a rabbit which survived 4 days after an intravenous inoculation of poliomyelitic streptococci. × 250.

FIG. 15. Focal necrosis and round cell infiltration. Cerebrum of a rabbit which survived 7 days after an intravenous inoculation of poliomyelitic streptococci. × 180.

PLATE 53.

FIG. 16. Perivascular infiltration. Cerebrum of a rabbit which survived 48 hours after an intravenous inoculation of non-poliomyelitic streptococci. × 105.

FIG. 17. Focal necrosis and round cell infiltration. Cerebrum of the same rabbit as Fig. 16. × 105.

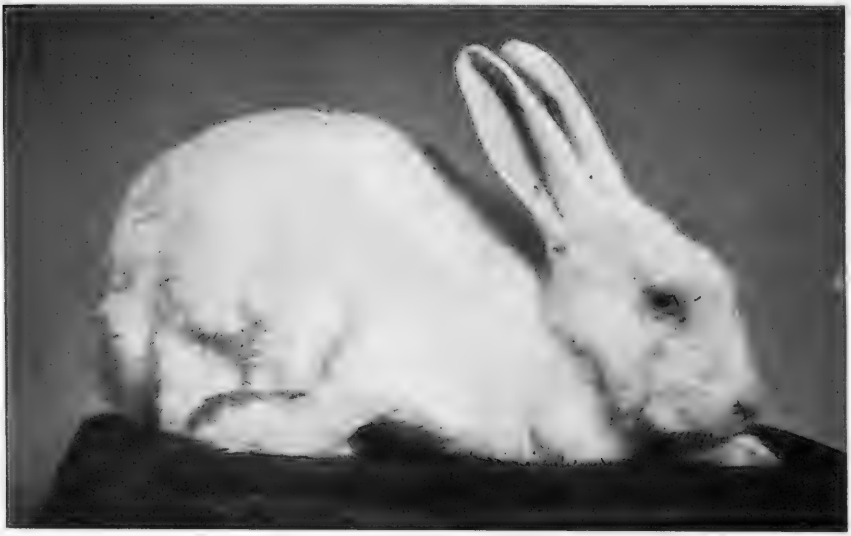

FIG. 1.

FIG. 2.

(Bull: Streptococci from Poliomyelitis.)

FIG. 3.

FIG. 4.

(Bull: Streptococci from Poliomyelitis.)

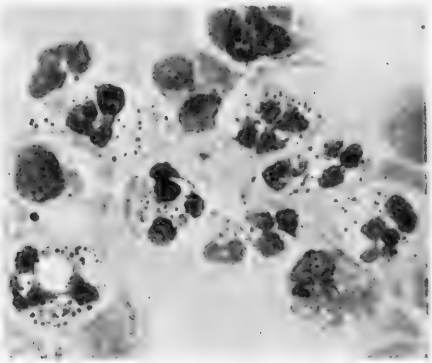

FIG. 5.

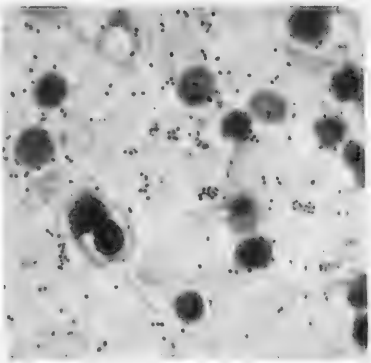

FIG. 6.

FIG. 7.

(Bull: Streptococci from Poliomyelitis.)

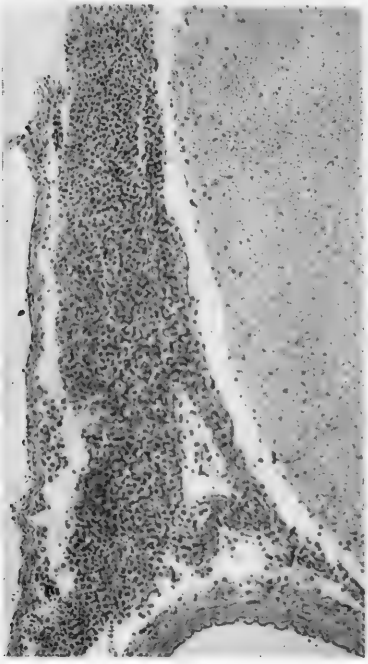

FIG. 8.

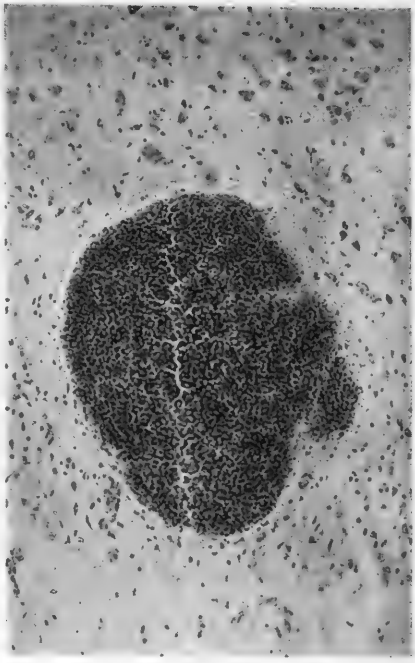

FIG. 9.

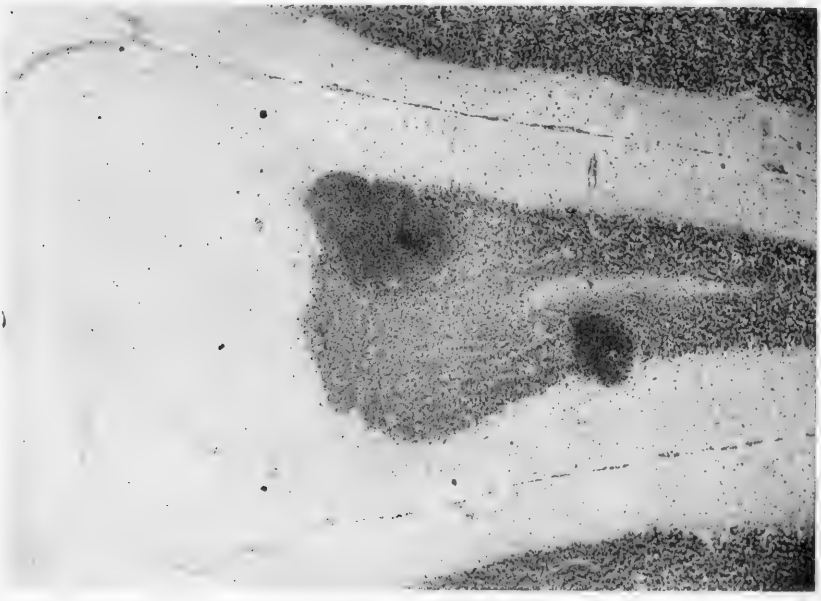

FIG. 10.

(Bull: Streptococci from Poliomyelitis.)

FIG. 11.

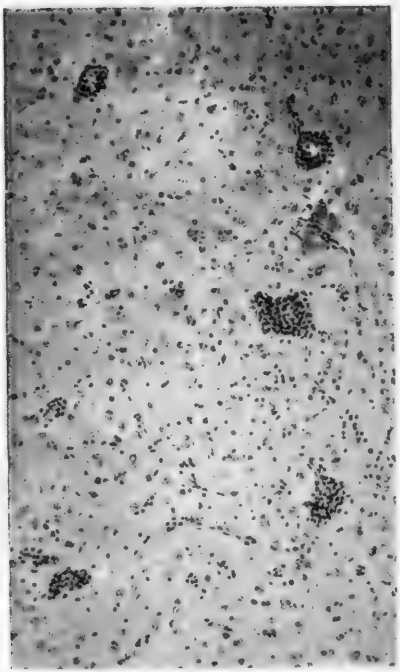

FIG. 12.

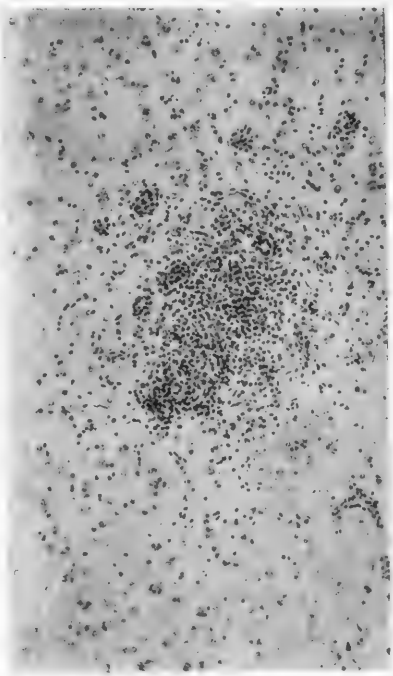

FIG. 13.
(Bull: Streptococci from Poliomyelitis.)

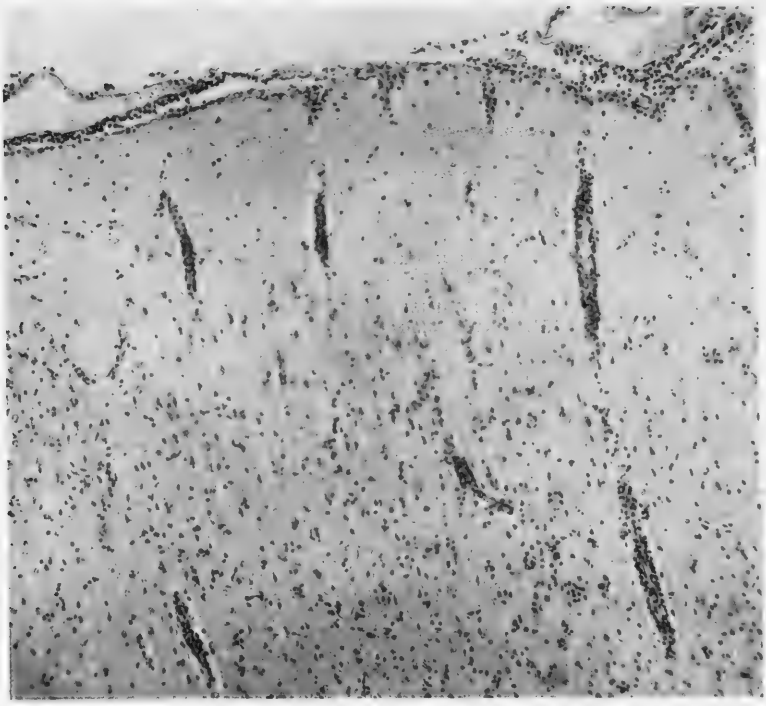

FIG. 14.

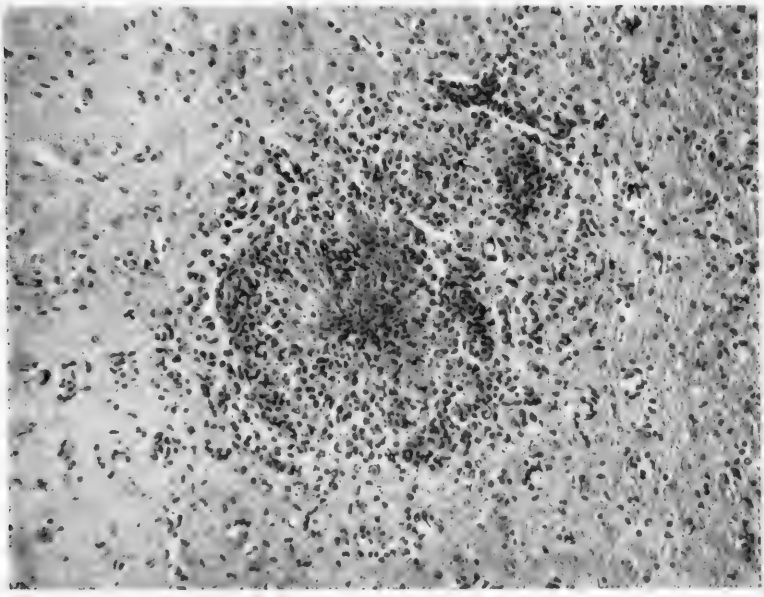

FIG. 15.

(Bull: Streptococci from Poliomyelitis.)

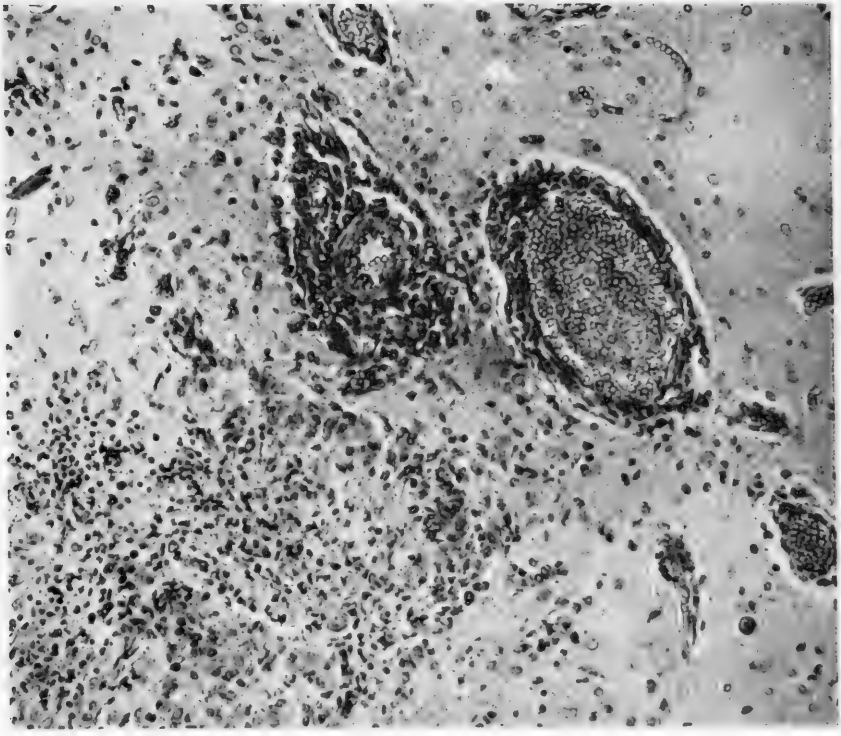

FIG. 16.

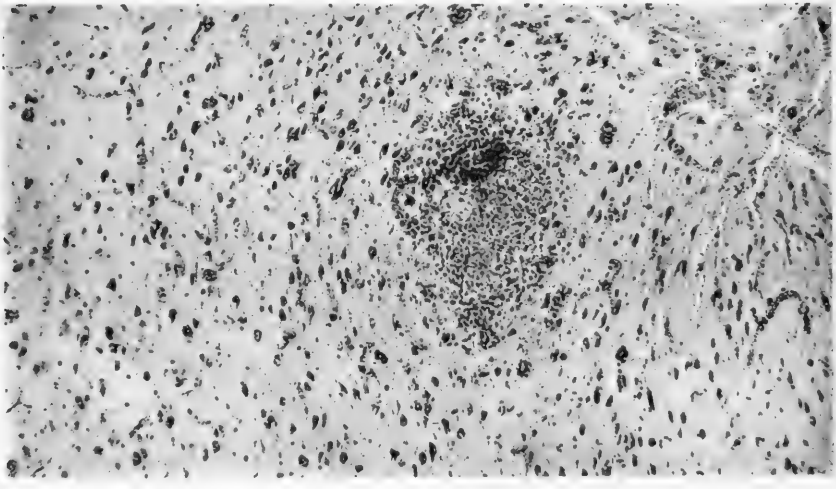

FIG. 17.

(Bull: Streptococci from Poliomyelitis.)

A REPORT ON THE SERUM TREATMENT OF TWENTY-SIX CASES OF EPIDEMIC POLIOMYELITIS.

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INTRODUCTION.

This report describes in some detail the method used and the results attained in twenty-six cases of acute poliomyelitis treated with human serum from recovered and convalescent cases of the disease. The cases were treated during the epidemic which prevailed in the summer and autumn of 1916 in New York State.

The epidemic was attended by a high death rate uniformly affecting large groups of cases occurring in city and suburban localities. In this, as in previous epidemics, the greater number of deaths occurred in those cases in which the upper portions of the spinal cord were involved, or in which an ascending, or Landry's type of paralysis occurred. The percentage of deaths from secondary causes, such as pneumonia, was small. Aside from this, no secondary or metastatic foci of infection, outside the central nervous organs, arose or were detected. This fact should be mentioned in connection with the claims that have been made that epidemic poliomyelitis is a form of streptococcus infection.[1]

At the onset the disease may offer a perplexing problem to the attending physician or expert diagnostician. In both the field and ward work at the Westchester Isolation Hospital use was made of lumbar puncture and microscopic and chemical examinations of the cerebrospinal fluid at the bedside in order to arrive at a rapid differential

[1] Mathers, G., *J. Infect. Dis.*, 1917, xx, 113. Rosenow, E. C., Towne, E. B., and Wheeler, G. W., *J. Am. Med. Assn.*, 1916, lxvii, 1202. Nuzum, J. W., and Herzog, M., *J. Am. Med. Assn.*, 1916, lxvii, 1205.

diagnosis. In addition to detecting two cases of epidemic cerebrospinal meningitis which quickly yielded to injections of the antimeningococcic serum, and one case of acute meningitis due to a bacillus probably belonging to the paracolon group, the procedure commended itself especially as it enabled us, first, to remove to the hospital cases on the 1st or 2nd day of illness, thereby contributing materially to the efficiency of the isolation, and, next, to bring under the specific serum treatment cases in the earlier stages of the affection. The groups of histories given show the advantages of the latter point, but it is mentioned here since it has been proved feasible, in field work in country districts, to take the necessary apparatus to the bedside and to make a diagnosis immediately by means of lumbar puncture and clinical examinations. Valuable time may be lost if the fluid is sent to the laboratory, and in the most perplexing cases the diagnosis can be made only by considering the character of the cerebrospinal fluid, as revealed by microscopic and chemical examination, in connection with the clinical findings at the time the fluid is withdrawn. In some cases, though rarely, it may be necessary to repeat the lumbar puncture in 6 hours.

HISTORICAL.

The discovery, almost simultaneously by Flexner and Lewis[2] and Landsteiner and Levaditi,[3] of the filterable nature of the microorganism, or virus, causing the disease, was quickly followed by Flexner and Lewis's[4] observation that recovery from an attack of experimental poliomyelitis afforded protection to a second inoculation; and this in turn was followed by the detection of immunity or neutralizing substances in the blood serum first of recovered monkeys and then of recovered human beings.[5] Since recovery from an attack of poliomyelitis was obviously brought about through a process of immunization, similar to that in other infectious diseases, Flexner and Lewis[6] endeavored to prevent the development of the

[2] Flexner, S., and Lewis, P. A., *J. Am. Med. Assn.*, 1909, liii, 2095.

[3] Landsteiner, K., and Levaditi, C., *Compt. rend. Soc. biol.*, 1909, lxvii, 592.

[4] Flexner and Lewis, *J. Am. Med. Assn.*, 1910, liv, 45.

[5] Levaditi and Landsteiner, *Compt. rend. Soc. biol.*, 1910, lxviii, 311. Römer, P. H., and Joseph, K., *Münch. med. Woch.*, 1910, lvii, 568. Flexner and Lewis, *J. Am. Med. Assn.*, 1910, liv, 1780. Anderson, J. F., and Frost, W. H., *J. Am. Med. Assn.*, 1911, lvi, 663.

[6] Flexner and Lewis, *J. Am. Med. Assn.*, 1910, liv, 1780.

infection in inoculated monkeys through the administration of blood serum taken (*a*) from recovered monkeys and (*b*) from recovered human beings. The results, while not constant and regular, were definite. The method pursued by Flexner and Lewis was first to make an intracerebral inoculation of the active adapted monkey virus, and then, about 24 hours later, to begin treatment by intraspinal injections of the immune serum. Their results were prevention of the onset of paralysis in some cases, and delay of the onset in others. Account should be taken, however, of the high potency of the adapted virus and the mode of its inoculation since the number of infections following was 100 per cent and the mortality among the paralyzed animals nearly as high. The experimental disease is, therefore, far more severe than the corresponding epidemic condition in man; and presumably it is less subject to favorable influence through therapeutic measures.

These experimental results were at once utilized as a basis of a serum therapy in man.[7] Netter and his associates[8] have reported a total of thirty-four cases of acute poliomyelitis which they have treated by the subdural method of injecting immune serum. They have undoubtedly established the fact that, as in the monkey, subdural injections intelligently carried out in man are safe. They believe further that they have proved them to be definitely beneficial or curative. They became increasingly convinced that the period of the disease at which the injections were made counted vitally, and they urged that the injections should be made as early as possible in the course of the infection.

During the past summer the serum treatment has been carried out in a number of different hospitals. Very few reports giving the circumstances and details of the methods and results have thus far appeared. But a number of important facts bearing on the subject have been acquired from clinical observation and experimental study. In the first place, a meningitis accompanied by a pleocytosis precedes in many instances the onset of paralysis. It is, therefore, one of the particular tasks of the attending physician to detect the disease in this early period when the treatment offers the greatest possibility of benefit. In the second place, the meningitis is attended by an increased permeability of blood vessels in the inflamed pia arachnoid. By reason of this increased permeability, it is possible for protein and other substances introduced into the blood to reach the subarachnoid spaces and probably the perilymphatic spaces in the nervous organs

[7] Netter, A., Gendron, A., and Touraine, *Compt. rend. Soc. biol.*, 1911, lxx, 625.

[8] Netter, A., *Bull. Acad. méd.*, 1915, lxxiv, series 3, 403. Netter, A., and Salanier, M., *Bull. et mém. Soc. méd. hôp. Paris*, 1916, xl, series 3, 299.

which are otherwise excluded. Hence, while the most direct route to the perivascular lymphatic and perineural spaces in the spinal cord may still be by way of the cerebrospinal fluid, this is not the only way since a blood route is also available. This blood route may lead directly to the interstices of the nervous tissues. Flexner and Amoss[9] ascertained that an aseptic meningitis set up by an intraspinal injection of horse serum opened the way to the nervous tissues for the virus of poliomyelitis introduced into the blood, often leading to infection and paralysis which a blood injection alone does not accomplish. These authors[10] also report that after the subdural injection of horse serum, immunity principles passively introduced into the blood pass into the cerebrospinal fluid. These facts indicate that in endeavoring to influence the course of epidemic poliomyelitis in human beings, the following conditions should be observed: (1) early and prompt diagnosis and treatment; (2) intraspinal injection of immune serum; (3) injection of immune serum directly or indirectly into the blood. The coincident injection into the blood is indicated not only by the pathology of poliomyelitis but also by the physical phenomena involved, since it may be assumed that the tendency will be for an equilibrium to be established between the serum in the subarachnoid space and that in the blood. Increasing directly the serum in the blood should diminish the rapidity of outflow of that in the meninges. It happens fortunately that protein substances introduced into the low levels of the subarachnoid spaces pass into the blood more slowly than when introduced at higher levels.[11] Furthermore, it is possible to administer larger amounts of serum by the blood than intraspinally. Finally, the serum employed should be collected from cases which have recently passed through an attack of poliomyelitis, and not many years after, as has been the practice of Netter and others. It is to be supposed that the serum will contain a greater amount of immune substances in this early period than after the lapse of many years. A test carried out with a sample of blood serum taken from a patient on the 6th day of illness showed that it already contained the neutralizing principles.[10]

[9] Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1914, xx, 249.

[10] Flexner and Amoss, *J. Exp. Med.*, 1917, xxv, 499.

[11] Dixon, W. E., and Halliburton, W. D., *J. Physiol.*, 1915–16, l, 198.

CASE HISTORIES.

The serum employed in the series of cases to be reported was obtained from human beings who had recently recovered from an attack of epidemic poliomyelitis and who, at the time the serum was taken, were still paralyzed. All the donors of the serum except two had been convalescent for 8 weeks or less. The two exceptions had passed through the disease 2 years before.

The use of the serum from recently recovered persons, otherwise healthy, involves no risk of transferring the microorganism of poliomyelitis. In the first place, the virus has never been detected in the circulating blood of human beings even in the first days of the disease.[12] In the second place, even if minimal quantities of the virus are present in the circulating blood, the presence of the immunity principles in the serum would bring about its neutralization during the 48 hour period following the collection of the serum, during which sterility is being determined prior to its use.

The serum administered was collected under aseptic conditions, and was used uninactivated and unpreserved. The conditions surrounding the treatment of this series of cases were so carefully controlled that no risk was taken. On the other hand, one can now state that inactivation of the serum by heating to 53°C. for ½ hour or by the addition of 0.2 per cent tricresol does not impair the protective power.

Mode of Administration.—For the reasons given, we departed from the methods formerly employed and injected the serum not only intraspinally but also into the blood. The quantity of serum which can safely be given to a child by intraspinal injection within a 12 hour period is obviously limited. In order to increase the quantity of immune bodies present in the fluids and tissues, even larger quantities were injected intravenously directly, or indirectly by way of the skin. The accompanying tables, although based on a relatively small number of cases, indicate the advantage of the larger volumes of serum administered. Some degree of selection was exercised in choosing cases for the treatment. The quantity of available serum was limited, and in view of the unproved value of the serum it was used in those

[12] Clark, P. F., Fraser, F. R., and Amoss, H. L., *J. Exp. Med.*, 1914, xix, 223.

cases in which the fever had not lasted over 48 hours; in a few instances the serum was given to patients who were critically ill, although the febrile period was of much longer duration.

For purposes of analysis and comparison, the cases treated with serum may be divided into two groups. The first group comprises the cases in which some degree of paralysis existed before the serum was administered; the second group includes the cases in which at the beginning of the treatment no paralysis could be detected by physical examination. The cases in the second group, however, were instances of poliomyelitis, since the clinical histories, general findings, and changes in the cerebrospinal fluid left no doubt on that point.

The first group of cases should be further subdivided into two classes: of these one class was under observation long enough for us to determine that the pathologic process in the spinal cord was extending and involving other regions; in the other class treatment was begun immediately in the home and continued after admission to the hospital. No information was, therefore, available to indicate whether the progress of the disease had already become arrested or not.

Group I. Cases Showing Paralysis at the Beginning of the Treatment.
Class I. Paralysis Extending.

Case 1.—Male; age 33 years.

Sept. 2, 1916. Malaise, headache, and vomiting. Sept. 3. Unable to bear weight on legs. Sept. 4. Admitted to hospital. Legs completely paralyzed. No tendon reflexes. Cerebrospinal fluid shows 280 cells per c.mm. and increased globulin. Bladder, bowels, diaphragm, abdominal and thoracic walls, and upper extremities normal. Sept. 5. Bladder paralyzed; no abdominal movement during respiration; using accessory muscles of respiration; deltoids weak; cyanosis.

On Sept. 5, 10 cc. of serum were injected intraspinally and 20 cc. intravenously. The cyanosis gradually increased, and death resulted from respiratory paralysis 10 hours after the injection. No obvious influence on the course of the disease was exerted by the serum.

Case 2.—Female; age 10 years.

Sept. 22, 1916. Headache, nausea, fever, and abdominal pain. Sept. 23. Temperature 103°F. Pain in back and in right leg; drags right foot. Sept. 25. Admitted to hospital. Temperature 102.6°F.; pulse 112; respirations 30. Neck and back muscles stiff; flaccid paralysis of both legs; bladder functions. Sept. 26, 6 p.m. Left deltoid paralyzed; dyspneic; employs accessory muscles of respira-

tion; no respiratory movement of abdominal wall; bladder paralyzed. Cerebrospinal fluid contains 270 cells per c.mm.; globulin increased.

On Sept. 26, 20 cc. of serum were given intraspinally and 60 cc. subcutaneously. Sept. 27, 8 p.m. Temperature 99.8°F.; pulse 102; respirations 30. Abdominal muscles move with respiration. Sept. 28. Abdomen moves freely; left deltoid responds but not yet normally; bladder inactive. Sept. 30. Bladder acting; no change in leg condition.

The history of the acute stage of this case readily divides itself into two periods. The first (a) takes in the time up to and including the paralysis of the legs, followed by a brief interval when (*b*) the abdomen, diaphragm, shoulder, and bladder became paralyzed. It was during the second period that the serum was administered. Coincidently with the administration there was no further progress, but rather a rapid recession of the recent paralysis; the older paralytic condition was not obviously affected. Whether the phenomena described are of the nature of cause and effect cannot certainly be determined.

Case 3.—Male; age 3 years.

Sept. 26, 1916. Fever and vomiting. Sept. 27. Difficulty in swallowing. Sept. 28. Admitted to hospital. Temperature 101°F.; pulse 112; respirations 38. Paralysis of muscles of deglutition. Sept. 29. Complete paralysis of right side of face. 12 m. Temperature 104.2°F.; pulse 138; respirations 62. Very ill. 2 p.m. Cerebrospinal fluid, 208 cells per c.mm.; globulin slightly increased.

On Sept. 29, 15 cc. of serum were administered intraspinally, 20 cc. intravenously, and 40 cc. subcutaneously. Sept. 30. Temperature 99°F.; pulse 102; respirations 68. Marked improvement in general condition but no change in the degree of paralysis of the face or muscles of deglutition. Before discharge the paralysis had almost disappeared.

Whether the prompt subsidence of the severe general symptoms in this case is to be ascribed to the serum treatment cannot be stated positively. The fact that no extension of the paralysis occurred in spite of the severe symptoms may or may not have been due to the treatment. But in this instance, as in Case 2, the paralysis was extending at the time the serum was given and no further extension occurred. It is obvious that in the first case of this series, no result was accomplished. It should be mentioned, however, that as compared with the more energetic treatment subsequently employed that case received a very small quantity of the serum, which was administered late in the course of the disease.

Class II. Paralysis Present.

Case 4.—Female; age 33 years.

Oct. 16, 1916. Chilly sensations; abdominal pain; difficulty in walking. Oct. 17. Stiffness of neck; legs weak. Oct. 18. Admitted to hospital. Temperature 103°F.; pulse 104; respirations 34. Neck and back stiff; weakness of muscles of abdomen and left leg. None of the muscle groups are completely paralyzed. Cerebrospinal fluid, 50 cells per c.mm.; globulin increased.

On Oct. 18, 20 cc. of serum were given intraspinally and 50 cc. intravenously. Oct. 19. Paralysis stationary. Oct. 21. Temperature 99.6°F. No extension of the paralysis.

The serum was administered in this case probably within 24 hours of the onset of definite paralysis, but whether or not it influenced the progress cannot be stated.

Case 5.—Male; age 2 years.

Sept. 13, 1916. Fever and headache. Sept. 18. After an apparently normal interval, vomiting occurred and the neck and back became stiff. Sept. 20. Weakness of legs noticed. Admitted to hospital. Temperature 98.2°F.; pulse 108; respirations 44. Neck and back stiff; face slightly asymmetrical; abdominal wall relaxed; no reflexes; partial paralysis of muscles of legs. Cerebrospinal fluid, 37 cells per c.mm.; globulin increased.

On Sept. 20, 5 cc. of serum were given intraspinally and 30 cc. subcutaneously. The paralysis did not extend, but there was no immediate improvement of the paralyzed muscles.

The treatment in this case was given probably at the end of the 4th day of illness at a time when the paralysis had already been arrested. Apparently nothing definite was accomplished. Case 6, a sister of this patient, should be used for comparison.

Case 6.—Female; age 4 years.

Sept. 19, 1916. Fever, headache, and constipation. Sept. 20. Temperature 101.4°F.; vomiting. Facial asymmetry. 11 p.m. Admitted to hospital. Temperature 102°F.; pulse 140; respirations 32. Neck and back stiff; slight left facial paralysis; weak hamstring and quadriceps muscles on both sides; knee jerks and Achilles tendon reflexes increased. Cerebrospinal fluid cloudy and contains 920 cells per c.mm.; globulin increased.

On Sept. 20, 10 cc. of serum were given intraspinally and 25 cc. subcutaneously. Sept. 22. No extension of paralysis. Sept. 23. Temperature normal.

In this case the serum was given within 30 hours of the onset of the symptoms and at a time when weakness of the muscles of the thighs

was appearing. The temperature became normal in about 60 hours and no extension of the paralysis ensued (Text-fig. 5). Within 2 weeks recovery of all the weak muscles was complete. The striking point in the case is the large number of cells in the cerebrospinal fluid when the serum was injected.

Case 7.—Female; age 2 years.

Sept. 12, 1916. Headache and fretfulness. Sept. 14. Admitted to hospital. Temperature 101.8°F.; pulse 124; respirations 28. Weakness of flexors and extensors of right hip and of gastrocnemius. Achilles tendon reflexes absent. Cerebrospinal fluid contains 99 cells per c.mm. and increased globulin.

On Sept. 14, 5 cc. of serum were injected intraspinally and 30 cc. subcutaneously. Sept. 15. Temperature normal; paralysis stationary. There was never any extension, but gradual improvement of the partially paralyzed muscles. The temperature curve is shown in Text-fig. 1.

Case 8.—Male; age 22 months.

Sept. 9, 1916. Child ill. Sept. 10. Admitted to hospital. Temperature 102.6° F.; pulse 136; respirations 36. Weakness of left quadriceps anterior.

On Sept. 10, 5 cc. of serum were administered intraspinally at home before removal to the hospital. Sept. 11. Left thigh muscles weaker. 20 cc. of serum given subcutaneously. Sept. 13. Temperature normal. Partial paralysis unchanged.

Case 9.—Female; age 3 years.

Oct. 12, 1916. Child feverish and irritable. Oct. 14. Left leg partially paralyzed. Oct. 15. Admitted to hospital. Temperature 99.8°F.; pulse 120; respirations 32. There is stiffness of the neck and back, weakness of the extensors of the left foot, and toe-drop. Cerebrospinal fluid contains 115 cells per c.mm.; globulin increased.

On Oct. 15, 15 cc. of serum were injected intraspinally and 40 cc. subcutaneously. The temperature fluctuated irregularly between 98.6° and 100° F. for 2 weeks. The weakness of the foot and leg increased somewhat. 1 month later some degree of impairment was present in the left quadriceps, hamstrings, peroneals, and toe extensors, while the anterior tibial group was completely paralyzed.

The serum was injected in this case within about 72 hours of the first appearance of any symptoms and at a time when only slight and limited paralysis existed. Nevertheless, the muscular impairment progressed slowly in the muscles first affected and into neighboring muscles, but the degree of paralysis never became severe.

Case 10.—Male; age 15 months.

Sept. 9, 1916. Child feverish and sweating; vomited. Admitted on same day. Temperature 99.6°F.; pulse 132; respirations 36. The neck and back are stiff

and there is doubtful weakness of the right deltoid. Cerebrospinal fluid shows 30 cells per c.mm.; globulin increased.

On Sept. 9, 5 cc. of serum were given intraspinally. There was no further involvement of the right deltoid and the temperature remained normal during the stay in the hospital.

This child was seen within a few hours of the onset of the first symptoms and the serum injection was given at the home before removal to the hospital. No paralysis developed.

Case 11.—Female; age 2 years.

Sept. 27, 1916. At noon the child was feverish. 10 p.m. Stiffness of the neck and back, weakness of the left quadriceps and peroneal muscles, and slight weakness of the intercostals. The patient, whose brother was already in the hospital, was admitted at once. Temperature 101°F.; pulse 110; respirations 32. Cerebrospinal fluid, 30 cells per c.mm; globulin increased.

On Sept. 27, 10 cc. of serum were given intraspinally and 30 cc. subcutaneously. Sept. 28. After 24 hours the temperature was 100°F.; no extension of muscular weakness. Sept. 29. Temperature normal. Muscular impairment gone. No abnormality on discharge.

The serum was administered to this child within 12 hours of the first appearance of the symptoms, and although a degree of weakness of certain muscles was already present, it quickly disappeared.

Case 12.—Male; age 3½ years.

Sept. 12, 1916. Headache and fever. Sept. 13. Temperature 103.8°F. Stiffness of neck and back; weakness of muscles of right ankle; toe-drop. Cerebrospinal fluid, 250 cells per c.mm.; globulin increased.

On Sept. 13, 10 cc. of serum were injected subdurally and 40 cc. subcutaneously. Sept. 14. Temperature 99.6°F. Weakness of muscles diminished rapidly.

The serum was administered within 24 hours of the onset of symptoms and at a time when several muscle groups were impaired. The temperature quickly fell to normal and the muscular weakness soon disappeared.

In considering Cases 1 to 12 the points that can be made definitely are: (1) the diagnosis was clearly established in each; (2) there was some grade of paralysis present in each; and (3) with the exception of Cases 1, 8, and 9, there was either no increase of the existing muscular weakness or paralysis, or there was prompt improvement in these conditions.

Group II. No Paralysis at the Time of Treatment.

In the following series of fourteen cases, no paralysis was detected up to the time that the serum was administered. One reservation should, however, be made: in the early stages of the disease, some of the patients were too ill to warrant a full examination of all the muscle groups and hence a degree of weakness or paralysis may have sometimes been overlooked. Moreover, the quantity of serum administered varied considerably, depending upon circumstances. The cases will be considered in the order of the amounts of serum given.

Case 13.—Male; age 8 years.

Sept. 19, 1916. Headache, fever, and drowsiness. Sept. 20. Same condition; neck and back stiff. Sept. 21. Admitted to hospital. No paralysis detected. Temperature 102.5°F.; pulse 102; respirations 32. Cerebrospinal fluid, 850 cells per c.mm.; globulin increased.

On Sept. 21, 5 cc. of serum were given intraspinally and 15 cc. subcutaneously. Sept. 24. Temperature normal. In the interim weakness of the following muscle groups developed: deltoids, pectorales major, rotators (outward) of arms, flexors of hip, quadriceps and hamstrings, and abdominal wall.

The serum was administered within 48 hours of the onset of symptoms. The amount given intraspinally was small. Weakness subsequently developed in many muscle groups, but in none was the paralysis complete.

Case 14.—Male; age 19 months.

Sept. 19, 1916. Vomiting, attributed to improper food. Sept. 20. Fever; neck and back stiff; muscular twitchings. Admitted to hospital. Temperature 103°F.; pulse 124; respirations 32. While there was muscular stiffness, no weakness was detected. Cerebrospinal fluid, 120 cells per c.mm.

On Sept. 20, 5 cc. of serum were injected intraspinally and 20 cc. subcutaneously. The fever persisted, and paralysis developed, involving ultimately the muscles of respiration. Sept. 23. Died.

Obviously no influence on the course of the disease was exerted by the serum. The experience gained later now indicates that the injection should have been repeated.

Case 15.—Female; age 4 years.

Sept. 23, 1916. Fever and headache; irritable. Sept. 24. Neck and back stiff; muscular twitching. Admitted to hospital. Temperature 103.6°F.; pulse

128; respirations 34. No muscular impairment detected. Cerebrospinal fluid, 360 cells per c.mm.; globulin increased.

On Sept. 24, 10 cc. of serum were given intraspinally and 20 cc. subcutaneously. Sept. 25. Flaccid paralysis of right arm and shoulder girdle with rapid extension and involvement of muscles of respiration. Died.

Autopsy showed severe poliomyelitic lesions of the cervical cord and medulla.

Obviously this case was not benefited by the serum injection, since paralysis developed a few hours after the treatment and extended rapidly.

Case 16.—Female; age 8 years.

Sept. 23, 1916. Headache; restless. Sept. 24. Temperature 102°F. Neck stiff. Cerebrospinal fluid, 730 cells per c.mm.; globulin increased.

Within 6 hours of the onset 10 cc. of serum were administered intraspinally and 20 cc. subcutaneously. The temperature fell slowly and reached normal on the 4th day. During this period weakness of the muscles of both arms and possibly slight weakness of the left leg appeared; complete paralysis was never present. Improvement was rapid, and almost complete recovery of lost strength was made in a short time.

Case 17.—Female; age 2 years.

Sept. 17, 1916. Fever; drowsy; muscular twitching; convulsions. Sept. 19. Admitted to hospital. Cerebrospinal fluid, 60 cells per c.mm.; globulin increased. Temperature 104.2°F.; pulse 132; respirations 32. Neck and back stiff; no muscular weakness.

On Sept. 19, 6 cc. of serum were given intraspinally and 25 cc. subcutaneously. Within 24 hours the temperature was normal. No muscular weakness developed.

Case 18.—Male; age 5 years.

Sept. 14, 1916. Temperature 103°F.; headache; drowsy. Sept 15. Neck and back stiff. Temperature 104°F. Admitted to hospital. Temperature 103.4°F.; pulse 126; respirations 50. No weakness of muscles. Cerebrospinal fluid, 379 cells per c.mm.; globulin increased.

Within 24 hours of the onset 5 cc. of serum were given intraspinally and 25 cc. subcutaneously. Sept. 16. Temperature 101°F. 5 cc. of serum were introduced intraspinally. Cerebrospinal fluid, 249 cells per c.mm. Sept. 17. Temperature normal. No muscular weakness ever developed.

Case 19.—Male; age 4 years.

Sept. 17, 1916. Temperature 102°F.; pain in back. Sept. 18. Temperature 102.4°F. Neck and back stiff. Cerebrospinal fluid, 150 cells per c.mm.; increased globulin. Admitted within 24 hours of onset.

On Sept. 18, 10 cc. of serum were injected intraspinally and 25 cc. subcutaneously. 15 hours later the temperature became normal. No muscular weakness developed.

Case 20.—Male; age 13 months. Cousin of Case 19.

Sept. 17, 1916. Drowsiness and fever. Sept. 18. Cerebrospinal fluid clear and contains 75 cells per c.mm.; globulin increased. The patient was given 10 cc. of serum subdurally and was shortly afterwards brought to the hospital. On admission, temperature 101.4°F.; pulse 120; respirations 26.

Immediately after admission a subcutaneous injection of 25 cc. of serum was given. The temperature rose to 104°F. and fell to normal 22 hours afterwards. There was no subsequent weakness or paralysis.

This patient was treated about 18 hours after the first symptoms. The temperature previous to treatment is not known. After a rise to 104°F. the temperature fell rapidly, reaching normal about 22 hours after the first injection (Text-fig. 2). The child developed no subsequent weakness or paralysis.

Case 21.—Female; age 3½ years.

Sept. 3, 1916. Headache; temperature 102°F. Sept. 4. Temperature 104°F. Sept. 5. Temperature 101.5°F. Stiffness of neck. Sept. 6. Cerebrospinal fluid clear and contains 230 cells per c.mm.; globulin increased. Admitted to hospital the same afternoon. On admission, temperature 102.4°F.; pulse 130; respirations 42. Stiffness of neck and back; no weakness or paralysis noted.

Immediately after admission 5 cc. of serum were injected subdurally and 40 cc. subcutaneously. Temperature reached normal 32 hours after treatment. No definite weakness or paralysis developed subsequently.

In this case treatment was begun about 3 days after the initial symptoms and at a time when paralysis had not appeared. Treatment was followed by a rapid drop in temperature and no weakness or paralysis developed during the period of observation.

Case 22.—Female; age 3½ years.

Oct. 7, 1916. Fatigue, irritability, and vomiting. Oct. 8. Fever and a mild delirium. Oct. 10. Fever persisted; drowsiness. Admitted to hospital. On admission, temperature 101.6°F.; pulse 132; respirations 28. Stiffness of neck and back. Clear cerebrospinal fluid with 88 cells per c.mm.

On Oct. 10, 20 cc. of serum were injected subdurally and 30 cc. subcutaneously. Temperature rose to 104°F. that night and there was slight nystagmus. Lumbar puncture yielded a slightly turbid fluid under considerable pressure. About 30 cc. of fluid were withdrawn from the spinal canal. On the following morning the nystagmus had disappeared and 36 hours after treatment the temperature reached normal. Cultures of the turbid fluid were sterile. Turbidity was due to the presence of a large number of polymorphonuclear leukocytes. No weakness or paralysis developed subsequent to treatment.

This patient was treated 3 days after the initial symptoms when there was no evidence of weakness or paralysis. Following the treatment there was evidence of increased irritation of the meninges and increased pressure in the subdural space which was relieved by lumbar puncture and withdrawal of fluid. The temperature reached normal 36 hours after the injection and the child did not develop any weakness or paralysis subsequently. The serum used in this case contained considerable fat; whether or not this played a part in the reaction following the injection cannot be stated definitely. This was the only instance in which any such reaction was obtained, although other cases were treated with portions of the same serum.

Case 23.—Male; age 5 years.

Oct. 3, 1916. Vomiting. Oct. 4. Fever; vomiting persisted. Clear cerebrospinal fluid with 250 cells per c.mm. Admitted to hospital. On admission, temperature 101°F.; pulse 130; respirations 30. Stiffness of neck and back; no weakness or paralysis.

15 cc. of serum were immediately given subdurally and 40 cc. subcutaneously. Temperature rose to 103°F. and fell to normal 12 hours later. There was no subsequent weakness or paralysis.

This patient was treated about 30 hours after what seemed to be the initial symptoms. At the time of treatment there was no demonstrable involvement of any muscle group. The temperature dropped to normal 12 hours after treatment and the child did not show any weakness or paralysis at any time.

Case 24.—Female; age 1½ years.

Oct. 11, 1916. Temperature 101°F. Oct. 12. Stiffness of the neck and back with muscle tenderness. Temperature 103°F. No demonstrable weakness. Admitted to hospital. On admission, temperature 103°F.; pulse 118; respirations 28. Clear cerebrospinal fluid with 40 cells per c.mm.; globulin increased.

On Oct. 12, 25 cc. of serum were injected subdurally and 35 cc. subcutaneously. Temperature dropped to normal within 16 hours after treatment. At no time was there any demonstrable weakness present.

This patient was treated about 30 hours after the mother first noticed fever, and at a time when there was no demonstrable weakness of any muscles. The temperature became normal in less than 24 hours after treatment (Text-fig. 3). An older sister of this patient was admitted to the hospital on the day previous to the one on which

this case was admitted. The sister had been sick for a period of 4 days with temperature as high as 104°F. She was admitted at the end of the febrile period, 4 days after the onset, and at the time of admission lumbar puncture showed the presence of a clear fluid with 76 cells and increased globulin. She received no serum and developed no weakness or paralysis.

Case 25.—Male; age 5 years.

Oct. 5, 1916. Irritability and fever. Oct. 6. Fever persisted; constipation. Oct. 7. Admitted to hospital. On admission, temperature 103°F.; pulse 104; respirations 28. Marked stiffness of neck and back; active reflexes; positive Kernig. No demonstrable weakness of any muscle group. Clear cerebrospinal fluid with 260 cells per c.mm.; globulin increased.

On Oct. 7, 22 cc. of serum were given subdurally and 45 cc. subcutaneously. The temperature fell to normal within 22 hours after treatment and the patient did not develop any weakness or paralysis.

This patient was treated about 48 hours after onset of the first symptoms and within 22 hours after treatment the temperature was normal (Text-fig. 4). No weakness or paralysis developed. A younger sister of this patient was admitted to the hospital 2 days previously with flaccid paralysis of both lower extremities. In this case onset had occurred 5 days before admission and the temperature had fallen to normal and paralysis had already set in when she was admitted.

Case 26.—Male; age 23 years.

Dec. 9, 1916. Vomiting and headache. Stiffness of neck, hyperesthesia, and asymmetry of patellar reflexes. Kernig's sign present on right side. Temperature 101°F. No demonstrable weakness of any muscle group. Clear cerebrospinal fluid with 40 cells per c.mm.; globulin increased.

38 hours after onset the patient received 20 cc. of serum subdurally and 100 cc. intravenously. Following treatment the temperature fell to normal within 48 hours, the hyperesthesia disappeared within that time, and no paralysis or weakness developed.

This patient, an adult, was treated within 38 hours after the initial symptoms appeared, at a time when there was no demonstrable involvement of the muscles. The temperature rapidly fell to normal, and no weakness or paralysis developed subsequently.

The more important facts relating to the twenty-six cases described are collected in Table I.

TABLE I.

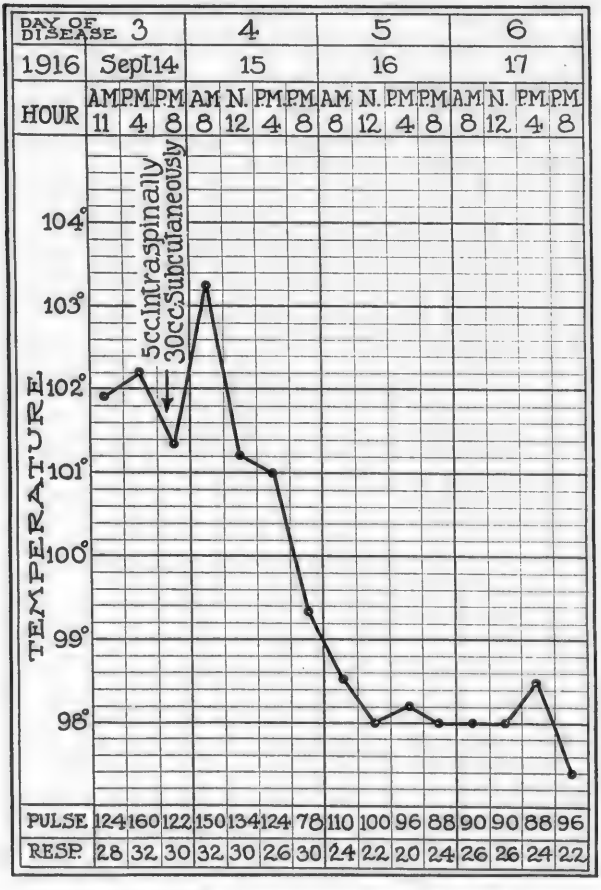
Summary of Cases That Received Serum Treatment.

Case No.	Age.	Clinical condition.	Spinal fluid.		Time between onset of disease and treatment.	Serum administration.			Temperature before treatment.	Time between treatment and normal temperature.	Total amount of serum given.	Result.
			No. of cells.	Globulin.		Subdural.	Subcutaneous.	Intravenous.				
	yrs.				*hrs.*	*cc.*	*cc.*	*cc.*	*°F.*	*hrs.*	*cc.*	
1	33	Ascending paralysis of legs, bladder, and abdominal and thoracic muscles.	280	++	72	10		20	101.2		30	Died 10 hrs. after treatment.
2	10	Ascending paralysis of legs, bladder, abdominal wall, diaphragm, left deltoid, and right facial muscles.	270	++	96	20	60		101.4	24	80	Deltoid and diaphragm functioning 24 hrs. later. Voided 4 days later.
3	3	Right facial and deglutition muscles paralyzed.	208	+	72	15	40	20	104.2	18	75	No further progress of paralysis. Subsequent improvement marked.
4	33	Weakness of left leg and abdominal muscles.	50	+	54	20		50	103.0	60	70	No progress of paralysis. Subsequent improvement.
5	2	Weakness of sternocleidomastoids, both legs, and intercostals.	37	+	96	5	30		98.2		35	No essential change.
6	4	Partial paralysis.	920	++	30	10	25		102.0	60	35	" increase. Complete recovery rapid.
7	2	Weakness of flexors of right thigh.	99	+	48	5	30		101.4	24	35	No progress of lesion. Subsequent improvement.
8	$1\frac{10}{12}$	Weakness of left quadriceps.			48	5	20		102.0	48	25	Slight increase in weakness.

9	3	Weakness of left anterior tibialis.	115	+	72	15	40		100.2	Low fever for 2 wks.	55	Definite increase in extent of paralysis of left leg.
10	$1\frac{3}{12}$	Weakness of right deltoid.	30	++	24	5			101.0	24	5	No increase in weakness.
11	2	Weakness of left peroneal and quadriceps and slight weakness of intercostals.	30	±	12	10	30		101.0	30	40	" " " paralysis. Rapid disappearance of weakness noted on admission.
12	$3\frac{1}{2}$	Slight right toe-drop.	250	++	24	10	40		103.0	24	50	No paralysis.
13	8	No paralysis. Left knee jerk diminished.	850	++	48	5	15		102.5	60	20	Developed partial paralysis in arms, legs, and abdominal muscles.
14	$1\frac{7}{12}$	No paralysis.	120		31	5	20		103.0		25	Died in 66 hrs.
15	4	" "	360	++	36	10	20		103.2		30	" " 28 "
16	8	" "	730	++	6	10	20		102.0	96	30	Slight partial paralysis of both arms. Subsequent recovery complete.
17	2	" "	60	±	36	6	25		102.0	22	31	Subsequent rise to 102°F. Variable temperature for 5 days. No paralysis.
18	5	" "	379	+	$22\frac{1}{2}$	10 (2 doses).	25		103.4	32	35	No paralysis.
19	4	" "	150	++	24	10	25		102.4	15	35	" "
20	$1\frac{1}{12}$	" "	75	++	18	10	25		101.4	22	35	" "
21	$3\frac{1}{2}$	" "	230	++	66	5	40		102.4	32	45	" "
22	$3\frac{1}{2}$	" "	88		76	20	30		101.6	36	50	" "
23	5	" "	250		30	15	40		101.2	12	55	" "
24	$1\frac{1}{2}$	" "	40	+	31	25	35		103.0	16	60	" "
25	5	" "	260	+	48	22	45		103.2	22	67	" "
26	23	" "	40	++	38	20		100	101.0	48	120	" " Hyperesthesia disappeared in 48 hrs.

DISCUSSION.

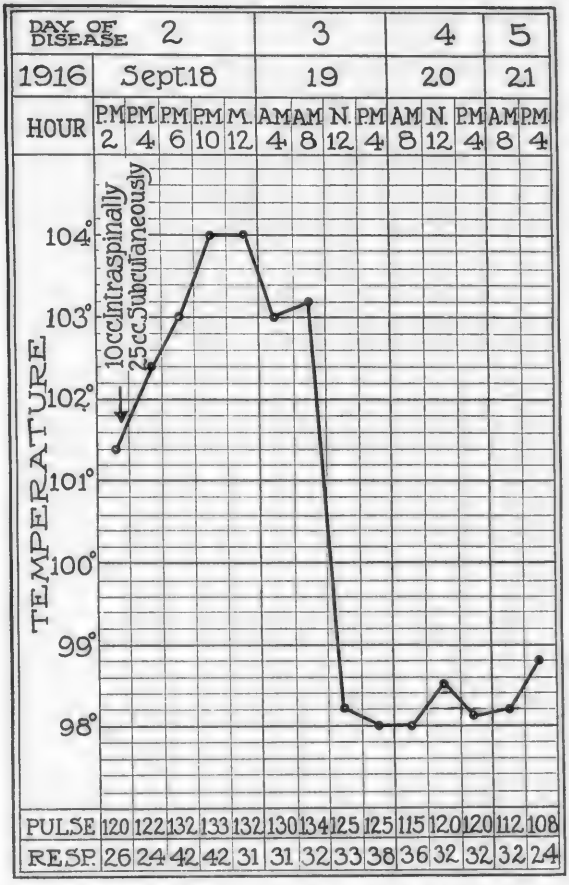
Since the data in the twelve cases which showed paralysis at the time serum was first given are less complete, and the control of the conditions was less perfect, we shall dismiss them with few comments.



TEXT-FIG. 1. Temperature chart of Case 7.

One patient died 10 hours after the serum was given, two patients suffered some degree of extension of the paralysis, while the remaining nine showed no extension of the paralysis.

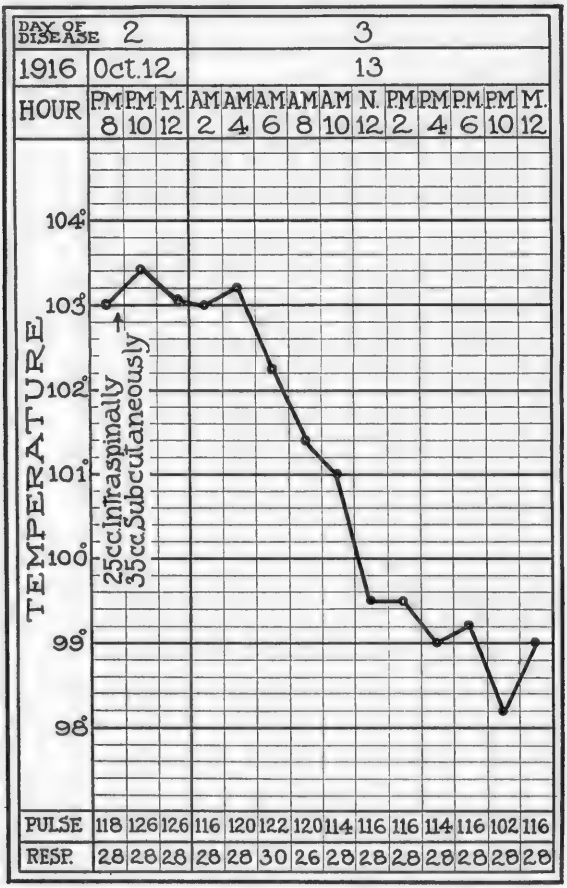
The circumstances surrounding the group of fourteen cases in which no paralysis was detected at the time serum was administered are more favorable for a conclusion. Two of the patients of this group developed respiratory paralysis and died; and two others de-



TEXT-FIG. 2. Temperature chart of Case 20.

veloped some degree of weakness or partial paralysis of certain muscle groups. The ten remaining cases (71 per cent) never showed any detectable weakness.

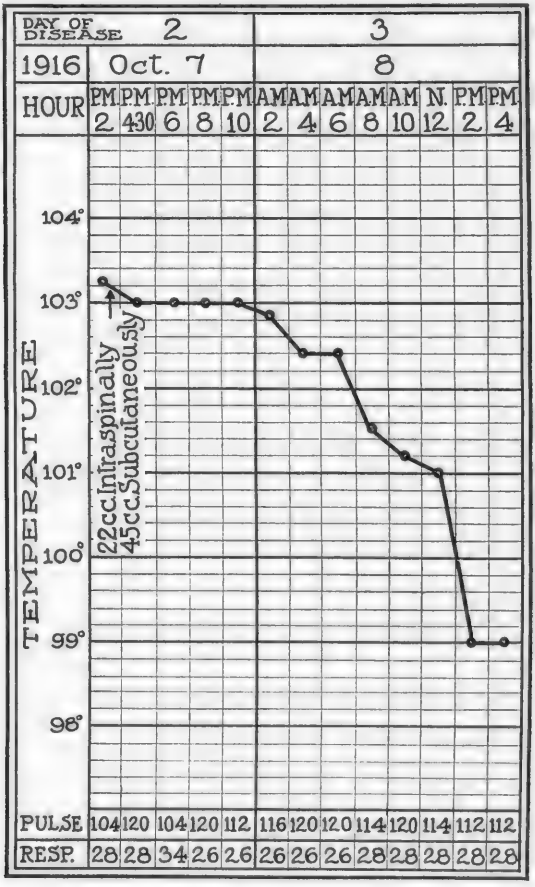
The analysis should be carried further. In the ten instances in which no paralysis occurred, the fever, sometimes high, tended to fall rapidly to normal, the average time of the fall being 25.7 hours.



TEXT-FIG. 3. Temperature chart of Case 24.

The critical fall in temperature is shown in the charts (Text-figs. 1 to 4). In this connection it is desirable to allude to two incidental effects of the intraspinal injection of an homologous serum. The

first is the following sharp rise in temperature, of brief duration; the second is the increase in stiffness of the neck and even the accentuation of the opisthotonos already present. The former has been



TEXT-FIG. 4. Temperature chart of Case 25.

frequently noted especially after the intraspinal injection of salvarsanized serum employed in the treatment of syphilis of the nervous system;[13] the latter results from the irritation or even transient asep-

[13] Ellis, A. W. M., and Swift, H. F., *J. Exp. Med.*, 1913, xviii, 428.

tic inflammation incited in the meninges by the serum. Coincident with this condition the cerebrospinal fluid may become turbid (Case 22) from emigrated leukocytes; but the inflammation lasts only a day or two and is in no way a source of danger.

In each case there was an increase in the number of cells in the fluid withdrawn by lumbar puncture as well as excess of globulin. In several instances the cell count was high (Cases 6, 13, and 16). In nine of the fourteen included in the second group, the count exceeded 100 cells per c.mm. As far as this is an index in pointing to the probable development of paralysis, it is favorable to the serum treatment.

This moderate number of cases is sufficient to demonstrate the harmlessness of the serum when introduced intraspinally under suitable conditions. The gravity method of injection was always employed. Each sample of serum was tested bacteriologically and found to be sterile; and in every instance a Wassermann test was employed to exclude syphilitic taint. Particular care was taken to obtain serum free from corpuscles or hemoglobin. Only perfectly clear and colorless samples were employed for injection. We believe that attention should be directed to these points in order that the reaction to the presence of the serum, a foreign body in the subarachnoid space, may be reduced to the minimum.

Finally, we had in mind that in acute poliomyelitis to a greater extent than in epidemic meningitis, the substance of the spinal cord and medulla suffer injury. On that account they may suffer even more readily the ill effects of increased intracranial pressure. In poliomyelitis the lumen of the blood vessels is already encroached upon by the perivascular infiltration. Hence the serum should be injected under low pressure and only after the previous withdrawal of a greater amount of spinal fluid than the serum injected. Should symptoms referable to increased intracranial pressure arise, they may be relieved by prompt withdrawal of the injected fluid. In one instance only (Case 22) in our series did we resort to the withdrawal as a precautionary measure, although the removal was not urgently demanded.

In estimating the probable effects of the serum other considerations

arise. The first relates to the period of the disease at which the serum is injected. If there is analogy between this disease and epidemic meningitis, for example, then the earlier the serum is injected, the more pronounced should be the effects. In other words, it should theoretically be easier to prevent paralysis altogether or to limit its extent and intensity than to bring about its rapid retrogression. This fact follows from the pathology of poliomyelitis[14] and from the manner in which automatic arrest of the pathologic process takes place.

The treatment was begun with definite plans for administering the serum early in the disease and in large amounts. The time limit between onset and treatment was set at 48 hours. It is obviously impossible to adhere strictly to this rule, but out of the twenty-six cases reported, eighteen were treated within this time. Apparently the best results are obtained in cases treated within 30 hours after onset, though beneficial results were obtained in one instance as late as 96 hours after onset (Table II).

The serum administered was obtained from persons recently recovered from poliomyelitis, at which period it is supposed to contain immune substances in greatest concentration. We suggest that if serum is used from patients whose attacks are more remote, correspondingly larger doses should be employed.

Recovery from poliomyelitis depends upon a process of self-immunization, and the indications are that the immunization takes place rapidly. On the 6th day of the disease, neutralizing immune bodies are already present in readily detectable quantities in the blood.[10] Therefore, what the therapeutic employment of the serum seeks to accomplish is the anticipation by artificial means of this process of autoimmunization. Probably also the artificial may have advantages over the automatic method. The latter operates chiefly by the medium of the blood, although doubtless the increased permeability of the meninges and choroid plexus in the damaged nervous organs permits an escape of immune bodies into the cerebrospinal fluid. It has been shown by direct experiment that the neutralizing immune bodies pass into the aseptically inflamed cerebrospinal fluid.[10] But

[14] Flexner and Lewis, *J. Exp. Med.*, 1910, xii, 227.

TABLE II.

Effect of Early Administration of Serum.

Case No.	Time between onset and treatment.	Result.	
	hrs.		
2	96	Marked improvement.	+*
5	96	No change.	0
22	76	" paralysis.	+
3	72	Marked improvement.	+
9	72	Increase in paralysis.	−
1	72	Died in 10 hrs.	−
21	66	No paralysis.	+
4	54	Improvement.	+
7	48	No increase in paralysis.	+
8	48	Slight " " "	−
13	48	" " " "	−
25	48	No paralysis.	+
26	38	" "	+
15	36	Died in 28 hrs.	−
17	36	No paralysis.	+
14	31	Died in 66 hrs.	−
24	31	No paralysis.	+
6	30	" "	+
23	30	" "	+
19	24	" "	+
12	24	" "	+
10	24	" "	0
18	$22\frac{1}{2}$	" "	+
20	18	" "	+
11	12	Improvement.	+
16	6	Slight increase in paralysis.	−

* In the tables + indicates improvement in the condition of the patient; 0 indicates no change; and − indicates that the disease was not checked.

it is generally conceded that the most direct route to the interstices of the central nervous organs is by way of the cerebrospinal fluid.[15] Hence the introduction of the immune serum intraspinally and intravenously at the same time should afford the quickest and most effective way of supplying the nervous tissues with the immunity substances which neutralize and inactivate the virus of poliomyelitis.

It has been stated, moreover, that larger amounts of serum should

[15] Flexner, S., *J. Am. Med. Assn.*, 1913, lxi, 447, 1872.

be administered in the early stages, in which the greatest benefit is expected. Table III shows that the best results are obtained in cases receiving a total of more than 30 cc. of serum.

TABLE III.

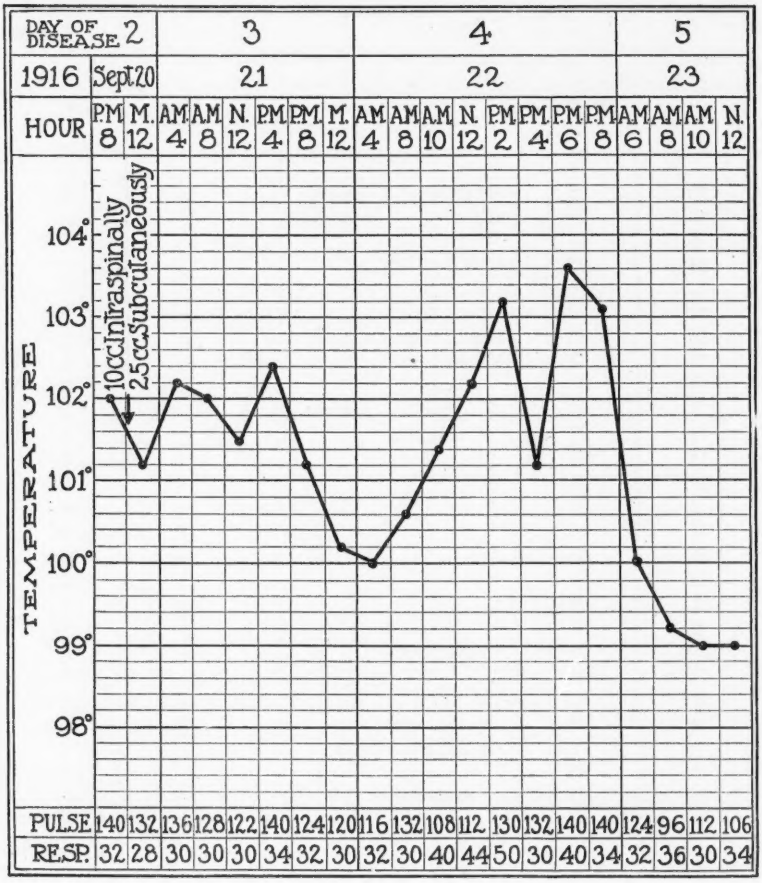
Effect of the Amount of Serum Administered.

Case No.	Total amount of serum given.	Result.	
	cc.		
10	5	No increase in weakness.	0
13	20	Slight " " paralysis.	−
8	25	" " " "	−
14	25	Died in 66 hrs.	−
1	30	" " 10 "	−
15	30	" " 28 "	−
16	30	Slight increase in paralysis.	−
17	31	No paralysis.	+
5	35	" change.	0
7	35	" increase in paralysis.	0
18	35	" paralysis.	+
19	35	" "	+
20	35	" "	+
6	35	" "	+
11	40	" "	+
21	45	" "	+
12	50	" "	+
22	50	" "	+
9	55	Definite increase in paralysis.	−*
23	55	No paralysis.	+
24	60	" "	+
25	67	" "	+
4	70	Improvement.	+
3	75	Marked improvement.	+
2	80	" "	+
26	120	No paralysis.	+

* Serum given 72 hours after onset of symptoms.

The patients who received a total of more than 30 cc. within 48 hours after the onset, without regard to the clinical condition, age, or temperature, seemed to be benefited in every case but one, and in this case the temperature quickly dropped to normal (Table IV).

It seems highly desirable, therefore, to administer at least 50 cc. of serum in every case and if possible to treat cases within 30 hours after the onset of the first symptoms.



TEXT-FIG. 5. Temperature chart of Case 6.

The plan of giving single large doses was strictly adhered to in this series, but a study of the temperature charts and clinical notes indicates that in some cases either larger doses or a second dose of serum

TABLE IV.

Results in Cases Treated within 48 Hours after Onset with More than 30 Cc. of Serum.

Case No.	Time between apparent onset and treatment.	Total amount of serum given.	Result.
	hrs.	*cc.*	
7	48	35	0
25	48	67	+
26	38	120	+
17	36	31	+
24	31	60	+
6	30	35	+
23	30	55	+
19	24	35	+
12	24	50	+
18	22½	35	+
20	18	35	+
11	12	40	+

should have been given. Case 6 received in all 35 cc. of serum and recovered rapidly without paralysis. However, the temperature chart suggests that a second dose of serum should have been given on the 4th day of the disease (Text-fig. 5).

CONCLUSIONS.

1. Serum taken from recently recovered cases of poliomyelitis may be employed in its treatment and probably yields the best results.

2. When sterile for ordinary bacteria, free of corpuscles and hemoglobin, and when injected by the gravity method, observing well known rules of caution, it may be employed without danger.

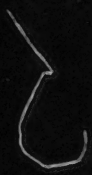
3. The serum should be injected both intraspinally and intravenously, the latter either directly or by way of the subcutaneous tissues.

4. The earlier in the course of the disease the serum is employed in suitable doses, the more promise there is of benefit.

5. The action of the serum appears to be more precise and definite in arresting paralysis than in rapidly bringing about its retrogression.

6. The decision to employ the serum should rest upon a clinical examination supported by the results of the microscopic and chemical study of the cerebrospinal fluid.

7. The question of multiple and repeated injections of the serum

has not yet been worked out. In the cases here reported and especially in the group in which no paralysis existed at the time of the first injection, the pathologic process either did not progress at all, or where there was extension, as in Cases 14 and 15, the upper segment of the spinal cord became rapidly involved, and was followed by respiratory paralysis and death. Probably in cases in which some degree of muscular weakness develops soon after the injection of serum, reinjection 12 to 24 hours later may be advantageous. The temperature curve may serve to indicate the time for reinjection.

8. The favorable results thus far achieved in human beings by means of the immune serum support and extend those obtained experimentally in monkeys and indicate, as was foreseen, that the milder or less fatal form of poliomyelitis appearing in man is even more amenable to the serum treatment than is the highly fatal disease produced by inoculation in monkeys.